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CYSTEINE AND RELATED COMPOUNDS FOR DIFFERENTIATING MEMBERS OF THE GENUS SALMONELLA[1,2]

W. R. HINSHAW[3]

INTRODUCTION

CERTAIN EUROPEAN INVESTIGATORS, including Manninger (1930),[4] Miessner (1930), Wagener (1934), and Haupt (1935), have recommended that *Salmonella pullorum* and *Salmonella gallinarum*[5] be classified as identical species. Although, undoubtedly, these bacteria are antigenically alike, poultry-disease investigators do not all concur with these four researchers, since the two organisms show certain biochemical and morphological differences and produce two distinct diseases.

Hinshaw and Rettger (1936), in attempting to determine additional biochemical differences between the two organisms, found that the 0.15 per cent cysteine gelatin devised by Valley (1929) for anaerobic culture furnished a medium for differentiating these organisms into two distinct species.

The present paper will describe additional studies on cysteine and related compounds as possible agents in differentiating closely related species of the *Salmonella* group. *S. pullorum* and *S. gallinarum* were

[1] Received for publication June 24, 1940.

[2] This paper covers the major portion of the work submitted to the Graduate School of Yale University by the author in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The experimental work reported herein was done by the author in the laboratories of the Department of Bacteriology, Yale University, and of the Division of Veterinary Science, University of California.

[3] Associate Professor of Veterinary Science and Veterinarian in the Experiment Station.

[4] See "Literature Cited" for complete dates on citations, which are referred to in the text by author and date of publication.

[5] The nomenclature of the Salmonella Subcommittee of the International Society of Microbiologists (1934) has been used throughout this publication.

taken as type species. Also studied were eighteen other species (or types) of the genus *Salmonella* together with biochemical variants of several strains of *S. pullorum*, and sixteen species belonging to other genera.

REVIEW OF LITERATURE

In the *Salmonella* group, most species are flagellated. The Kauffmann-White scheme, adopted by the Salmonella Subcommittee (1934) for differentiating species, provides a satisfactory method for this group. *S. pullorum* and *S. gallinarum* (both nonmotile) cannot, however, be differentiated by antigenic means because of their identical somatic antigen. The review of literature here concerns principally the differentiation of *Salmonella* species by other than antigenic means; *S. pullorum* and *S. gallinarum* have received special attention. The reviews are arranged, as far as possible, according to specific means of identification.

Salmonella gallinarum was first described as *Bacillus gallinarum* by Klein (1889), who isolated it from an acute outbreak of infectious enteritis in adult fowl. Moore (1897) described a similar organism, *Bacterium sanguinarum*, from a disease of adult chickens which he called "infectious leukemia." Since then the two organisms have been proved identical—and the same as *Bacterium typhi gallinarum alcalifaciens* reported by Pfeiler and Rehse in 1913. All of these authors regarded the organism as the cause of a definite adult fowl disease.

Rettger (1900, 1901) first described *Salmonella pullorum* as the causative agent of a fatal ailment of baby chicks now universally known as pullorum disease. He called it *Bacterium pullorum*. Many pathologists throughout the world, after investigating this condition, have accepted it as a disease principally of young chicks (or other young birds). A monograph by Rettger and Plastridge (1932) gives an extensive bibliography of this chick epizootic and its causative organism.

As stated above, one distinct difference between *Salmonella pullorum* and *S. gallinarum* is the former's predilection for causing acute losses in young birds, and the latter's for producing acute symptoms principally in adults. There are, however, exceptions. Jones (1913) described an acute outbreak of pullorum disease in adult chickens. Similar reports have been issued by Gwatkin (1927), Bunyea (1927), Edwards and Hull (1929), Tittsler (1930), Plastridge and Rettger (1930, 1934), and Stafseth (1931).

Fowl typhoid of young birds has been reported by several investigators—Beaudette (1925), Beach and Davis (1927), Martinaglia (1929), and Komarov (1932). Hinshaw and Taylor (1933), having isolated *Salmonella gallinarum* from the ovary of a turkey, suggested possible

transmission through the egg. Beaudette (1925, 1930) has recorded isolations from chicken eggs laid by carriers of *S. gallinarum*. Gauger (1934) isolated *S. gallinarum* from the testes of a chronic carrier of fowl typhoid. Thus the criteria for considering these two species as differentiated by predilection for age or by method of transmission are less reliable than was once contended.

Smith and TenBroeck (1915*a*, 1915*b*) were among the first to present a comparative study of *Salmonella gallinarum, S. pullorum,* and *S. typhi*. Their studies included agglutination affinities, toxin-producing properties, and carbohydrate reactions. They noted the antigenic likeness of the three species. According to them, *S. gallinarum* and *S. pullorum*, though alike in many respects, are different enough to be considered separate species. The basis for this conclusion is the production of gas by most strains of *S. pullorum* and the fermentation of maltose by *S. gallinarum* but not by *S. pullorum*. Other comparative studies on these organisms include those of Rettger and Koser (1917), Goldberg (1917), and Dumaresq (1938).

Mulsow (1919*a*, 1919*b*) first noted maltose fermentation of certain strains of *Salmonella pullorum* and suggested dulcitol as a better differential compound. This maltose fermentation has also been noted by Krumwiede and Kohn (1917), Spray and Doyle (1921), Beaudette, Bushnell, and Payne (1923), Edington (1924), Bushnell, Hinshaw, and Payne (1926), Hendrickson (1927), and others. According to Hendrickson, all differences in the fermentation reactions between *S. pullorum* and *S. gallinarum* disappear when serum water is used as a basic medium. Under these conditions, Hendrickson maintains, the only essential difference between the two organisms is the more vigorous growth of *S. gallinarum*. Beaudette (1930) concurs with him (as well as with others) that this greater vigor is a differential feature of *S. gallinarum*. He also observes that the organism has a disagreeable odor, not noticed in *S. pullorum* cultures. According to Edwards (1928), acid production in maltose by *S. pullorum* results from slow alkalinization of the medium during long incubations and from subsequent hydrolization of this sugar by the alkali formed.

Rodrigues and Pacheco (1936*a*) though failing to confirm Edwards' results on the hydrolysis of maltose, found in serum an enzyme that hydrolyzes maltose. They, therefore, recommend caution in using serum water as a stock medium for studying maltose fermentation.

More recent literature, besides presenting several reports of maltose-fermenting strains of *Salmonella pullorum,* confirms Mulsow's recommendation that dulcitol, not maltose, be used to differentiate between the two species. Van Roekel (1935) reported a laboratory strain of *S. pul-*

lorum which decomposed maltose after several years as a nonfermenter of this sugar. Later (1937) by colony selection he obtained stable variants that subsequently fermented maltose after even a few hours' incubation at 37° C.

Hadley, Caldwell, Elkins, and Lambert (1917) were prompted by the occurrence of both aerogenic and anaerogenic strains of *Salmonella pullorum* to classify the former as the alpha type and the latter as the beta. Weldin and Weaver (1929) reviewed the literature on gas production. Of one hundred strains of *S. pullorum* studied by them, eight produced no gas at any temperature. The others varied in their aerogenic properties, some producing gas at all temperatures, others only at temperatures varying from 25° to 37° C.

Except possibly in the Duisburg strains (Müller, 1933), *Salmonella gallinarum* appears more stable in its reactions than *S. pullorum*. Some investigators would probably consider the Duisburg type a maltose-fermenting variant of *S. pullorum,* because it failed to decompose sodium potassium tartrate (Kauffmann, 1934) or to produce the "gallinarum" reaction in cysteine gelatin (Hinshaw and Rettger, 1936). It does ferment dulcitol, however, and has other characteristics of *S. gallinarum.*

Type B of *Salmonella intermedius* is a maltose-fermenting strain, considered by Nobrega (1935) to be a variety of *S. pullorum* because it reacts in tartrate medium and because it is lysed by *S. pullorum* bacteriophage.

Various differential media have been suggested for separating these two species. Cruickshank (1927) utilized Andrade's lactose agar for original streaking from tissues. If growth occurs in 12 hours at 37° C, if the lactose is not fermented, and if the growth consists of Gram-negative rods, it is inoculated into a double-sugar medium containing 1 per cent maltose, 0.1 per cent glucose, and Andrade's indicator. *Salmonella gallinarum* produces a permanent deep-red color in both the slant and the butt of the tube in 18–24 hours. *S. pullorum* usually produces gas and a faint pink color in the end of the tube, the slant remaining colorless.

Mallman and Snyder (1929) used dextrin-lactose agar and dulcitol-lactose agar for differentiation. These media consist of 2 per cent basal agar containing 1 per cent dextrin (or dulcitol) and 0.5 per cent lactose with bromthymol blue as an indicator. With *Salmonella gallinarum* the top was blue, and a yellow color appeared in the butt; *S. pullorum* produced no change; *Escherichia coli* produced a yellow-to-blue top and a yellow butt; and *Pasteurella avicida,* a blue top and a green butt.

The use of brilliant green in media is limited largely to its value as an inhibitory agent for certain contaminants, but allows *Salmonella*

species to multiply. Mallman, Thorp, and Semmes (1928) reported variation in the bacteriostatic action with different members of the *Salmonella* group. They did not, however, propose that this property be used to differentiate species.

Kerr (1930) suggested that brilliant green agar (1:100,000 dilution) may aid in differentiating *Salmonella gallinarum* and *S. pullorum* on original isolation because of the difference in colony growth. The former grows readily, producing a larger colony, with a brownish tinge. Telluric acid added to brilliant green agar (to a dilution of 1:25,000) causes colon types to produce colonies that are "brilliant green fast" to be darkened and readily distinguishable from others.

Delpy and Rastegar (1938) suggest that brilliant green may be useful in two ways to differentiate species of *Salmonella:* first, through utilizing the property of some bacterial species of reducing the dye; second, through variation in bacteriostatic action. These authors also reviewed the principal means of distinguishing *S. pullorum, S. gallinarum,* and the closely related species or varieties. The following media or reactions were suggested by them for differentiation purposes: xylose, maltose, amidon, dulcitol, and sorbitol broths, Jordan-Harmon tartrate agar, neutral-red fluorescence, gas production, and brilliant-green reduction. The use of neutral red solution for detecting gas formers by the fluorescence reaction is the same method as reported by Pacheco and Rodrigues (1935*a*). The neutral red medium consists of nutrient agar plus 1 per cent neutral red (8 parts per 1,000), covered with vaseline.

Beck and Eber (1927) give as additional differential methods the decomposition of Drigalski medium with the production of blue-gray color, and a change of Gassner medium to yellow by *Salmonella gallinarum.* The changes produced by *S. pullorum* are not specified, nor the kind of Gassner medium used.

Gressel (1928), studying twenty-nine stock cultures, found that sixteen conformed to *Salmonella pullorum* and three to *S. gallinarum* (*Bacterium paradysenteriae*), while ten apparently stood between these species. The slime-ring activity of *S. gallinarum* on Gassner's agar is emphasized somewhat as a differential characteristic for these two species.

Pacheco and Rodrigues (1935*a,* 1935*b,* 1935*c,* 1936*a,* 1936*b*) and Rodrigues and Pacheco (1936*a,* 1936*b*), in several papers on the biology of the pullorum-gallinarum group, compared the well-known types with an intermediate type, later called *Salmonella intermedius* type B. (Delpy and Rastegar, 1938). One paper (Pacheco and Rodrigues, 1935*b*) also discusses type A of *S. intermedius* of Delpy and Rastegar. The neutral-red fluorescence test for gas formers, the use of a modified Drigalski medium in which maltose distinguishes the maltose fermenters from the

nonmaltose fermenters, and the employment of a modified Pacheco and Mello (1932) medium for H_2S determination are the principal differential methods of these investigators. Their bismuth carbonate agar was modified by adding 0.01 per cent cystine. This gave stronger reactions with *S. gallinarum*, the only member of the group that reacted in either medium. Stress is laid on the production of H_2S for differentiating between the *S. intermedius* type B and the regular types. Even in lead acetate agar, the *S. intermedius* type B produces only a faint reaction.

Lovell (1932) summarized the important literature on the serological identification of *Salmonella* species; also included in a table are the most important fermentation reactions of the group. The historical background that has led to the antigenic classification of this group by Savage and White (1925*a*, 1925*b*), White (1926), Kauffmann (1929, 1930), and Kauffmann and Mitsui (1930) is amply discussed in Lovell's paper. White and his co-workers, and Kauffmann and associates, used essentially the same methods of differentiation—namely, by the types of flagellar and somatic antigens possessed by the organism. The Salmonella Subcommittee of the International Society of Microbiologists (1934) has now combined the Kauffmann and White systems into a single one called the Kauffmann-White scheme, and accepted by the originators of the two individual systems. Embodying as it does certain points of each individual system, it is now in international use for differentiating between species. The Kauffmann-White scheme, with a table of the important species and their antigenic formulas, is also given by Topley and Wilson (1936) and by Bergey (1939).

The use of specific bacteriophages in identifying members of the *Salmonella* group was suggested by Burnet (1929) when he demonstrated the close relation between the sensitiveness of particular phages to the distribution of particular somatic antigens. These relations seemed specific for antigenic groups, but not for species within the groups. Naidu (1935) described bacteriophagic differentiation of *S. pullorum* and *S. gallinarum* with a strain of bacteriophage which lysed the former, but which failed to attack the latter. His findings were confirmed by Nobrega (1935) and Munné (1937).

The results obtained by Naidu and by Nobrega contradict the observations of Pyle (1926) and Mallman (1931*b*). Pyle found no bacteriophages specific for *Salmonella pullorum*, and Mallman observed that bacteriophage from one organism could be easily adapted to another by the use of mixed cultures. Judging from the results reported, bacteriophage would differentiate somatic antigenic groups; also, a highly specific bacteriophage like that of Naidu and Nobrega could, if once isolated, perhaps be used in identifying species.

Organic acids have been employed less commonly for differentiation purposes in the *Salmonella* group than in the coli-aerogenes group. Koser (1923), exhaustively reviewing the earlier work, showed that results had not been systematically applied to the differentiation of bacteria. In that paper and in later ones (1924*a*, 1924*b*, 1926) he reported on the ability of members of the coli-aerogenes group to utilize various organic acids. On the basis of utilization he employed several of these substances for differentiation, especially between fecal and nonfecal types. The most satisfactory of these is citric acid.

Weldin and Miller (1931) studied 84 strains of *Salmonella pullorum* for their ability to utilize citric acid or sodium citrate, using the citric acid medium of Koser (1924*a*) and the citrate agar of Simmons (1926). They state:

> In Koser's citric acid medium 13 strains grew consistently, 10 failed to grow at all, and the remainder were variable in their reaction. In Simmons' citrate agar 21 grew in every test, 20 never grew, and the remaining 43 were variable. It was found difficult to determine in many instances whether growth had actually occurred or not in Koser's citric acid medium. The turbidity was so slight in many instances that results could only be recorded as questionable growth. On the other hand, growth in Simmons' citrate agar was slow but the reaction was definite if incubation was continued long enough. The majority of the strains of *S. pullorum* were shown to be able to utilize citric acid or sodium citrate as a sole source of carbon, although a few strains were found which were unable to attack these compounds. Neither medium is recommended for dividing this species into subgroups nor in the identification of *S. pullorum*.

Brown, Duncan, and Henry (1924) reported extensively on the fermentation of organic-acid salts as an aid in differentiating bacterial species. A large group of the salts of open-chain organic acids was tried in a concentration of 1 per cent of the basic medium of ordinary nutrient broth having a reaction of pH 7.4. Phenol red served as an indicator, and Durham's tubes for recording gas formation. Twenty different species, including thirteen belonging to the genus *Salmonella* (*S. gallinarum* and *S. pullorum* among them), were studied. Of the salts tested, those of formic, citric, and d-tartaric acid were the most satisfactory.

The d-tartrate medium gave the most regular reaction. *Salmonella typhi* produced an early and marked acidity, followed by definite alkalinity after 48 hours. *S. paratyphi* A caused little or no change in reaction, while *S. paratyphi* B produced a slight alkalinity with no preceding acid or gas. *S. paratyphi* C gave an acid reaction similar to *S. typhi* but produced gas. Neither *S. pullorum* nor *S. gallinarum* is mentioned. Because of the irregular gas production, the method was considered less reliable than the usual "sugar" fermentation method.

By means of a lead acetate solution, these same authors could deter-

mine whether or not organisms could utilize these salts; and much of their paper concerns the development of the technic of the lead acetate precipitation test. They employed thirteen strains (not including *Salmonella pullorum* and *S. gallinarum*) in these studies, which revealed constant results with individual species. By using six organic salts, they obtained seven different groupings of the common *Salmonella* types, whereas sugar reactions divided these same species into only four groups.

Jordan and Harmon (1928), employing the d-tartrate medium of Brown, Duncan, and Henry (1924), confirmed the observation that the Schottmüller and "aertrycke" types can be differentiated in this medium. All the Schottmüller strains of the Jordan collection failed to ferment sodium d-tartrate after 7 days' incubation, whereas the majority (78 out of 87) of the "aertrycke" types fermented this chemical within 48 hours. These investigators described a new medium composed of 2.0 per cent nutrient agar, 1.2 per cent of a 0.2 per cent alcoholic solution of phenol red, and 1.0 per cent sodium potassium tartrate, adjusted to pH 7.6–7.8. This medium, employed in tubes, unslanted, and inoculated by the stab method, surpasses Brown's medium. The paratyphoid strains that impart an acid reaction give a distinct yellow color in the lower portion of the tube and a red surface zone of 4–7 mm. The strains that produce an alkaline reaction give a diffuse homogeneous reddish discoloration. This medium has come into wide usage; it is mentioned often as a means of differentiating various *Salmonella* species. Topley and Wilson (1936, pages 552–53) list all the *Salmonella* known by that date, and include the reactions in this medium.

Mallman (1931*a*) tested several sodium salts of organic acids to determine their possible value in differentiating *Salmonella pullorum* and *S. gallinarum*. Two of these, the salts of mucic and d-tartaric acid, had merit. When grown in their presence, *S. pullorum* gives an alkaline, and *S. gallinarum* an acid, reaction. Mallman also refers to a personal communication relating how Henry and Duncan, after publishing their 1924 paper, found that *S. pullorum* fails to utilize d-tartrate, but will utilize the l-tartrate. *S. gallinarum,* in contrast, utilizes the d-tartrate, but not the l-tartrate. Hinshaw and Rettger (1936) confirmed Mallman's observations.

The ability of *Salmonella* species to produce H_2S from peptone and other media has been used for differential purposes by many investigators. Most references to H_2S production concern the use of Jordan and Victorson's (1917) lead acetate agar; and the results, especially for *S. pullorum* and *S. gallinarum,* are variable. Both these species have been reported as producing H_2S, although *S. gallinarum* has been the

more variable of the two. Tittsler (1931) reported a possible correlation of the incubation temperature to H_2S production; he found temperatures above 34° C to be inhibitory.

Pacheco and Mello (1932) proposed to use a semisolid agar plus bismuth carbonate as a medium for studying H_2S production. Pacheco and Rodrigues (1936*b*) used this medium, as well as a modification of it made by adding 0.01 per cent cystine.

Their findings indicate a dependence on the medium used for H_2S production. Their results with the cystinized Pacheco and Mello medium resemble those observed by Hinshaw and Rettger (1936) with cysteine gelatin and reported in more detail in the present investigation.

MATERIALS AND METHODS

Cultures from as many sources as possible were collected. These include old stock laboratory cultures, newly isolated organisms, and strains considered as variants. These, with their origin and history, are summarized under "History of Cultures."

In every instance standard chemical agents were used. Three brands of chemically pure cysteine hydrochloride have been used with equal success. Other chemicals used included the following: l-cystine, dl-methionine, glutothione, sodium potassium tartrate. The grades of gelatin used were: granular Difco-Bacto; Eastman's de-ashed purified gelatin; and ice cream gelatin. The agar, peptone, and beef extract used were all of the Difco-Bacto brands.

Careful records were kept on each new lot of medium, and each was given a lot number. These records included the brand of ingredients used, the date of making, the amount, and the results of check tests.

Preparation of 0.15 Per Cent Cysteine Hydrochloride Gelatin.—The formula for making the stock medium of cysteine hydrochloride gelatin, used in these studies, is as follows:

Peptone	5 grams
Beef extract	3 grams
Gelatin (granulated)	125 grams
Water (distilled)	1,000 cc

The gelatin base is prepared by the standard procedure. To it is added slowly 1.5 grams of cysteine hydrochloride. It may be dissolved first, in 25 cc of hot distilled water, if desired. This reduces the pH to between 5.0 and 6.0, so that one must correct it to 7.2 with an alkali such as KOH or NaOH. The medium is then tubed in 5- or 10-cc amounts, and sterilized in the autoclave for 20 minutes at 15 pounds' pressure. This medium has been kept as long as two months in a refrigerator without losing its differential characteristics.

Other Media.—All other media used were made up by the standard procedure, unless otherwise noted. All carbohydrates employed for fermentation studies were sterilized by filtration and were added aseptically to sterile peptone water containing bromthymol blue as an indicator. Jordan-Harmon sodium potassium tartrate agar was also used as a routine medium, in all the tests on the experimental media; it was prepared according to Jordan and Harmon (1928), and all inoculations were made by the puncture method.

Routine incubation of all cultures at 37° C for 72 hours was practiced, except in the experiments conducted to compare the results of incubation at different temperatures.

Care of Stock Cultures.—Stock cultures, when not in regular use, were maintained by seeding in 0.5 per cent nutrient meat-extract agar by the stab method, incubating at 37° C for 48 hours, sealing, and storing at room temperature in a dark compartment. Cultures sealed carefully have been kept as long as one year. As a routine procedure, however, transfers were made every 6 months, and the strains in use were transferred at least once a month.

Verification of Cultures.—All cultures, regardless of source, were studied to determine the authenticity of the species. These studies included their morphology, motility tests (by the hanging drop, and the semisolid agar method of Tittsler and Sandholzer, 1935), and cultural characteristics. Routine carbohydrate media included 0.5 to 1.0 per cent solutions of the carbohydrate in 1 per cent peptone water with bromthymol blue as indicator. The following fermentable substances were used, at all times, for routine studies: dextrose, lactose, maltose, sucrose, and dulcitol. Other fermentable media were used when deemed necessary. Antigenic studies were another means of obtaining data on specificity.[6]

HISTORY OF THE CULTURES[7]

Cultures of *Salmonella gallinarum* were secured from as many animal sources and organs as possible; they include turkey, chicken, guinea fowl, and human strains.

Most of the strains were isolated in California from chickens or turkeys. Strains were also obtained from Connecticut, Virginia, North Carolina, Massachusetts, Rhode Island, and New Jersey, as well as from Europe. European strains include the Duisburg variety of *S. gallinarum* (Kauffmann, 1934). The Yale stock culture collection strains include

[6] Determinations were made by Dr. P. R. Edwards, University of Kentucky.

[7] The complete history of all cultures used is tabulated in table I of the author's dissertation filed in the Yale University Library. In the text only the author's identification numbers are given.

G42, which is probably the original strain isolated and described by Moore (1897). G78 and G79, two guinea fowl strains, have been described by Beaudette (1938).

The Duisburg strains (G55, G56, G85) were obtained by Müller (1933) from cases of acute gastroenteritis in man. G57 was isolated from the potato and egg salad which is believed to have been the cause of the gastroenteritis. Kauffmann (1934) who studied these strains, designated them as the Duisburg variety of *Salmonella gallinarum* because of slight differences in the fermentation reactions. Antigenically they are identical with *S. gallinarum* of avian origin.

Representative strains of *Salmonella pullorum* from different sections of the United States, Europe, and South America, and isolated from various species of birds and other animals, are included in these studies. Classified according to animal origin, there are included in the regular stock collection 38 strains isolated from young chicks; 23 from adult chickens; 56 from turkey poults; 26 from adult turkeys; 3 from turkey eggs; 10 from ducks; 2 from pheasants; and 1 each from sparrows, pigeons, and foxes. The remainder come mostly from chickens or chicken eggs.

Various examples of biological and biochemical variants were secured to determine whether or not they react differently in the various media. These variants include 36 anaerogenic strains.

One strain of Pacheco and Rodrigues's (1935*a*) *Salmonella intermedius* type A, and two strains of their *S. intermedius* type B were furnished by Dr. Paulo Nobrega of the Instituto Biologico, São Paulo, Brazil. These strains are considered by Pacheco and Rodrigues to be intermediate types between *S. pullorum* and *S. gallinarum*. Their type A, a gas producer, ferments maltose and dulcite, but not xylose, and gives no reaction in Jordan and Harmon's (1928) tartrate agar. Their type B, a non-gas-producer, gives the same biochemical reactions as type A. According to Nobrega (1935) these organisms are probably varieties of *S. pullorum* because they are tartrate-negative and *S. pullorum* bacteriophage-sensitive. These types are further discussed in a recent paper by Delpy and Rastegar (1938).

Three dulcitol-positive strains, which in the present studies did not decompose maltose except when incubated for three weeks, were furnished by Dr. D. D. Nai, Perugia, Italy. One of these, P214, (Nai's strain 7), is described by Kujiumgieff (1937);[8] the other two, P217 and P218 (Nai's 15 and 21), are described by Barboni (1937). These variants have not been the subject of complete biochemical studies by the present writer, but they have been checked to determine whether they give con-

[8] Quoted in the correspondence by Nai.

sistently the variant reactions described by the donors. In addition, their reactions to cysteine hydrochloride gelatin and Jordan-Harmon tartrate agar have been investigated. The results appear in table 5 (p. 611).

Twenty-seven aerogenic strains which ferment maltose within 72 hours and which are dulcitol-negative have been encountered. These were isolated from California outbreaks or secured from Van Roekel (1935).

Fourteen representative strains of *Salmonella typhi* were used in these studies. All are of human origin except one, which is a strain described by Emmel (1936). According to him this strain was isolated from the intestine of a chicken. It has all the biochemical characteristics of the human strains. Two strains were dulcitol-negative strains in these studies, one is a nonmotile variant.

Sixteen species (or types) of *Salmonella* other than *S. pullorum*, *S. gallinarum*, and *S. typhi* have been tested one or more times in cysteine hydrochloride gelatin. A total of 148 strains is included, all of which have been antigenically typed according to the Kauffmann-White scheme. They include species isolated from chickens, turkeys, chukar partridges, ducks, pigeons, geese, foxes, swine, and horses. The *S. enteritidis*, *S. paratyphi* A, and *S. paratyphi* B strains are of human origin and are from the stock collections of the University of California and of Yale University.

Miscellaneous cultures of other genera include 12 species and from 1 to 5 strains of each (table 2, p. 598).

CYSTEINE HYDROCHLORIDE GELATIN AS A DIFFERENTIAL MEDIUM

Hinshaw and Rettger (1936) reported preliminary results on the discovery that *Salmonella gallinarum* produces a yellowish-white turbidity in a 12 per cent gelatin medium containing 0.15 per cent cysteine hydrochloride, after 48 to 72 hours' incubation at 37° C. *S. pullorum*, in contrast, caused no visible change in this medium even after prolonged incubation. The present work grew out of this earlier observation.

The reaction seen in *Salmonella gallinarum* cultures will be hereafter called the gallinarum reaction. It may be described briefly as follows: In 24 hours a slight cloudiness is usually noted in the upper part of the medium. In 48 hours this cloudiness increases both in intensity and in depth. By this time it is yellowish white or grayish and may extend throughout the upper half of the medium. The amount of reaction in 48 hours varies, and in some cultures may even be absent. Individual islands of flocculation may be present. In 72 hours, all cultures studied have shown turbidity, usually extending over the upper two thirds of

the column, with the lower third clear. By this time a slight amount of granular precipitate may be seen in the bottom of the tube, and sediment in the gelatin is usually blackened.

A total of 91 cultures of *Salmonella gallinarum* have been tested in cysteine hydrochloride gelatin. The 437 separate trials made with these were done over a period of nearly two years, with intervals of from a few days to several months between trials. During the intervals the cultures were stored in semisolid nutrient agar at room temperature. All but two organisms (G87 and G89) gave the typical gallinarum reaction in cysteine gelatin after incubation at 37° C for from 48 to 72 hours. While neither of these two exceptional strains was positive in cysteine hydrochloride gelatin, both gave an acid reaction in tartrate agar. These also differ from the type strains of *S. gallinarum* in that G87 is xylose- and dulcitol-negative, and that G89 is maltose- and arabinose-negative (table 5). Individual strains vary in the rapidity of the reaction and in the amount of precipitate. Usually the speed and the turbidity may be correlated with the intensity of the organism's growth on agar slants. Two strains, neither of which has ever grown well on meat-extract agar, have consistently been slow in producing the typical reaction and have had less turbidity than the other strains. When the cultures are removed from the incubator and left at room temperature (with the gelatin solidified), the medium clears progressively, beginning as early as two days after removal from the incubator. This clearing usually starts at the surface and extends over the entire area for a few millimeters below the surface. It progresses downward along the sides and toward the center, the central portion being the last to change. This tendency to clear varies greatly with individual strains and also with different lots of media. No explanation for the clearing has been found, but apparently the precipitate is dissolved. The yellowish-white granular precipitate that settles out does not redissolve. Strips of lead acetate paper suspended over the medium during incubation at 37° C become blackened within 24 hours, indicating a rapid liberation of H_2S. Little or no blackening of lead acetate paper has been noted in cultures of *S. pullorum* or in the uninoculated medium tested simultaneously with *S. gallinarum*.

The results obtained by seeding four strains of the Duisburg variety of *Salmonella gallinarum* (G55, G56, G57, and G85) resembled those with *S. pullorum*. In the 32 trials made with these strains, no turbidity has been noted even after prolonged incubation at 37° C. One reason why Kauffmann (1934) classified this strain as a variety of *Salmonella gallinarum,* rather than as the true species, was its failure to ferment the tartrate medium of Jordan and Harmon (1928). The present studies have verified Kauffmann's findings. The reaction produced by the Duis-

burg strains are more nearly like those of *S. intermedius* B of Delpy and Rastegar (1938), than any of the other variants studied.

A total of 454 different strains of *Salmonella pullorum* were studied, and a total of 808 trials were made. In one of the groups there were 160 cultures and 470 trials, or an average of 2.9 per strain. These trials were made over a period of two years, with intervals of from a few days to several months between trials. During the intervals, the cultures were stored in semisolid nutrient agar at room temperature. With but one exception, all of these strains gave negative reactions in the 0.15 per cent cysteine hydrochloride gelatin in 72 hours; the one tended to produce a slight flocculation, but no permanent turbidity. This culture grew somewhat more vigorously than the others, and the present interpretation of the atypical result is that the flocculation resulted from growth and not from any change in the medium. Incubation of *S. pullorum* in this medium for several days at 37° C or at room temperature produced no marked changes. The only change noted was the tendency of a few cultures to become slightly turbid at the surface after standing at room temperature for a few days. There were no indications that this medium may be useful in segregating the different species variants.

Fourteen strains of *Salmonella typhi* were studied and 49 trials were made. During the intervals between testing, the cultures were stored in nutrient semisolid agar at room temperature. This organism, like most paratyphoid strains, grows very profusely in this medium, so that the turbidity due to growth may be confused easily with the gallinarum reaction. This is especially true in the first 48 hours of incubation at 37° C. At this early period a flocculent type of colony growth often appears in the upper half of the column. When the tube is shaken, a turbidity results that is indistinguishable from the reaction. After 72 to 96 hours—sometimes even 48 hours—the cells settle, and the medium becomes clear or only slightly turbid. For this reason, final readings should not be taken for at least 72 hours. A heavy surface growth is also apt to appear in these cultures. None of the 14 strains has at any time given a positive reaction.

Miscellaneous Species of Salmonella.—Sixteen other species (or types) of *Salmonella,* received from diagnostic laboratories and from a few other sources, have been tested. Results with these cultures, together with those described above, are summarized in table 1. The results indicate that cysteine gelatin as used here is most useful as a means of separating *S. gallinarum* from *S. pullorum* and only of limited use for separating other strains in this genus. Since, however, this latter phase of the present work is only preliminary, many more strains and species must be studied before a definite conclusion is reached. It was difficult

to distinguish the gallinarum reaction from the turbidity due to growth. Most of the strains grew profusely and formed flaky islands in the body of the medium and heavy surface growths (pellicles). In some cultures this flaky growth settled on standing, which left a surface growth and a typical bacterial deposit in the bottom of the tube. In others (questionable reactions) the cloudiness remained, although it was less marked than with *S. gallinarum*. No strains found gave consistently the strong positive reaction seen in all the *S. gallinarum* cultures.

TABLE 1

SUMMARY OF REACTIONS OF *Salmonella* SPECIES IN CYSTEINE GELATIN

Species	Distribution of strains according to the reactions				Total strains
	Negative	Pellicle	Questionable	Positive	
S. pullorum	453	0	1	0	454
S. gallinarum	2	0	0	89	91
S. gallinarum var. *duisburg*	4	0	0	0	4
S. typhi	6	0	8	0	14
S. anatum	0	2	1	0	3
S. bareilly	10	2	0	1	12
S. bredeney	0	2	0	0	2
*S. california**	3	4	0	0	7
S. cholerae suis var. *kunzendorf*	6	5	0	0	11
S. derby	3	0	0	0	3
S. dublin	6	2	0	0	8
S. enteritidis	1	0	0	0	1
S. give	1	0	0	0	1
S. newington	2	0	0	0	2
S. newbrunswick	0	2	0	0	2
S. oranienburg	2	4	0	1	7
S. paratyphi A	0	0	0	2	2
S. paratyphi B	2	0	0	0	2
S. typhimurium	4	39	6	18	67
S. typhimurium var. *copenhagen*	9	4	0	2	15

* See: Edwards, Bruner, and Hinshaw (1940).

Miscellaneous Species of Other Genera.—Sixteen species of genera other than *Salmonella* were tested in the cysteine gelatin medium. The results of the preliminary trials of twelve of these are summarized in table 2, together with whatever histories were available.

Besides the species included in the table, a few other miscellaneous organisms were tested. With seven strains of *Escherichia coli* types isolated from turkeys, there was no regularity of results. Three gave no reaction, one a positive, and three a partial reaction. These strains, though not studied completely, consistently fermented dextrose, lactose, and maltose, but not sucrose. Four gave an acid reaction in Jordan-Harmon tartrate agar; three failed to do so. One of the last three gave a positive reaction in cysteine gelatin, a medium in which the other two

were negative. Three strains of *E. communior* types were also irregular—one being negative, one positive, and one partial in the cysteine medium. All fermented Jordan-Harmon tartrate agar. Similar irregular results were obtained with seven slow lactose fermenters. In the cysteine gelatin, two of these were negative, and five positive. All seven failed to ferment the tartrate agar.

A small Gram-positive coccus, often isolated from turkey poults, may

TABLE 2

Reactions of Miscellaneous Species in Cysteine Gelatin

Species	Distribution of strains according to the reactions			Total strains	History
	Negative	Questionable	Positive		
Escherichia coli	0	2	0	2	Yale stock strains 3 and 4
Brucella abortus	2	0	0	2	1 Yale, 1 California strain
Brucella suis	2	0	0	2	1 Yale, 1 California strain
Brucella melitensis	1	0	0	1	1 California strain
Shigella dysenteriae	1	0	0	1	Yale 21—Flexner type
Shigella dysenteriae	1	0	0	1	Yale 22—Shiga type
Phytomonas cerasi	4	0	0	4	Cherry gummosis strains
Pasteurella avicida	6	0	0	6	From rabbits and turkeys
Pseudomonas aeroginosa	0	2	2	4	From turkeys
Proteus species	0	3	2	5	From turkeys. All produced acid and gas in dextrose but no reaction in lactose, maltose, or sucrose broth
Corynebacterium peridicium	1	0	0	1	From quail; see Morley and Wetmore (1936)
Staphylococcus albus	1	0	0	1	From ovary of a turkey

at first be confused with *Salmonella pullorum* because of similar colony morphology. Four strains of this organism were negative in cysteine gelatin and in tartrate agar. Two strains of a Gram-positive diplococcus and two of a Gram-positive staphylococcus gave similar reactions.

Three of six strains of a Gram-positive streptococcus gave positive reactions, while two of them were negative in the cysteine-hydrochloride gelatin medium. Two of the three strains which gave the gallinarum reaction failed to ferment the Jordan-Harmon tartrate agar and one gave a positive fermentation reaction in this medium. The other three strains produced no reaction in the cysteine gelatin and failed to grow in the tartrate agar.

No comment on the reactions recorded in the table is necessary. Too few species are represented; and, except in the *Brucella* cultures, no very closely related species were studied. It was hoped that the cysteine gelatin might prove useful for differentiating the various species of the *Brucella* group; but, after two strains each of bovine and swine types

and one of human type had been tried, with negative results, no more were tested.

Recapitulation.—The results obtained by inoculating various species of bacteria into the 0.15 per cent cysteine hydrochloride nutrient gelatin are summarized in tables 1 and 2, which give the reactions in this medium for 20 species of *Salmonella,* 3 of *Brucella,* and 9 miscellaneous species. This medium definitely differentiates *S. gallinarum* from *S. pullorum, S. typhi,* and *S. enteritidis.* By its use, two strains each of *S. paratyphi* A and *S. paratyphi* B were separated. *S. paratyphi* A gave a positive reaction (though less marked than *S. gallinarum*), while *S. paratyphi* B gave a negative reaction.

All types of *Brucella* organisms gave a negative reaction, which eliminates this as a differential medium for members of this genus. The remaining species do not show differential possibilities, although the numbers of related species and trials are too small to warrant definite conclusions.

EFFECT OF VARYING THE PERCENTAGE OF CYSTEINE HYDROCHLORIDE

To determine the effect of varying the amounts of cysteine hydrochloride on the gallinarum reaction, the 12 per cent nutrient gelatin, pH 7.2, was kept constant, while the amount of cysteine hydrochloride was varied from 0.019 to 0.45 per cent. From 8 to 10 cc of medium were placed in 16-mm tubes, inoculated with *Salmonella pullorum* or *S. gallinarum,* and incubated at 37° C; readings were made after 24, 48, and 72 hours. All of the cultures were then held at room temperature for one week, after which a loopful of each culture was seeded on nutrient meat-extract agar to determine whether growth had been retarded by the cysteine hydrochloride. No retardation was noted with the different amounts of cysteine used. The results with *S. gallinarum* are summarized in table 3. To conform with previous records, only the 72-hour readings are noted. No further changes were observed after one week's incubation at room temperature. From 25 to 47 trials with *S. pullorum* and from 1 to 10 trials with *S. typhi* were negative in all the concentrations. *S. gallinarum* showed marked differences in the various concentrations of cysteine hydrochloride. Negative results were obtained in the 0.019 per cent concentration. Even though the results of nineteen strains seeded in the half-strength medium (0.075 per cent) are recorded as equal to those of the regular (0.15 per cent) double and triple strengths, there was a noticeable difference. No differences, except a possible slight increase in the yellowish-white precipitate, could be discerned between the 0.30, the 0.45, and the 0.15 per cent cysteine tubes. After 24 and 48

hours' incubation, the various lots showed little noticeable difference, although the 0.075 per cent tubes tended to show turbidity somewhat sooner than the more concentrated media. Judging from these studies, the optimum concentration of cysteine hydrochloride, from the standpoints both of cost and of efficiency, is 0.15 per cent, the amount which has been used in all of the routine experiments reported herein.

EFFECT OF VARYING THE CONCENTRATION OF GELATIN

In these trials all the ingredients except gelatin were kept constant. The various percentages of gelatin used were as follows: 2.5, 5.0, 6.5, 10.0, 12.5, 15.0, and 20.0. The organisms studied were *Salmonella gallinarum, Salmonella gallinarum* var. *duisburg, Salmonella pullorum, Salmonella typhi,* and *Shigella dysenteriae.*

TABLE 3

Influence of Varying Concentrations of Cysteine Hydrochloride on the Reaction with *Salmonella gallinarum*

Per cent concentration of cysteine hydrochloride	Distribution in the various grades of reaction*					Total number of trials
	4	3	2	1	Negative	
0.15	53	3	0	0	0	56
0.45	25	0	0	0	0	25
0.30	24	1	0	0	0	25
0.075	19	17	8	0	0	44
0.038	0	0	3	7	15	25
0.019	0	0	0	1	24	25

* In the grades, 4 indicates a typical gallinarum reaction; lower numbers indicate gradations in turbidity.

Except in the 20.0 per cent and 2.5 per cent gelatin tubes, *Salmonella gallinarum* gave typical reactions in the different concentrations. In the medium containing 20.0 per cent gelatin twenty out of twenty-three strains failed to give as pronounced a reaction as was noted in the regular (12.5 per cent) medium. The reaction was also slightly retarded in the 15 per cent gelatin; but at the end of 72 hours' incubation, it equaled that in the 12.5 per cent tubes. In the 6.5 and 5.0 per cent concentrations good reactions were obtained, but part of the turbidity appeared to result from growth. This was likewise true in the 2.5 per cent gelatin. In the 2.5 and 5.0 per cent tubes, settling occurred more rapidly than in the others; and, as might be expected, there was more yellowish-white precipitate.

No reactions were noted in the 20.0, 15.0, 12.5, and 10.0 per cent gelatin tubes inoculated with *Salmonella pullorum.* Concentrations under 10.0 per cent showed a tendency toward slight turbidity caused by the

growth of the organism and not by a cysteine reaction. This was equally true of every other *Salmonella* species. The two *Shigella dysenteriae* strains were negative in all concentrations.

Three strains of *Salmonella typhi* gave negative reactions in the 15.0 and 20.0 per cent gelatin media, whereas the other tubes showed some cloudiness. Although inconclusive because of the small numbers used, this result suggests that the greater viscosity of the higher concentrations of gelatin may prevent dispersion of the organism throughout the medium, and that growth of the organism, and not the cysteine reaction, causes the slight turbidity often seen with some species.

Prolonged incubation of the *Salmonella gallinarum* cultures at room temperature produced no changes that would materially affect the results recorded above. In the 2.5 to 6.5 per cent gelatin series, there was a rapid clearing after the maximum clouding of the medium. This apparently resulted, not from an actual disappearance of the insoluble material, but rather from a settling out, as indicated by a slightly greater amount of yellowish-white precipitate in these tubes. In the higher-concentration series, there were larger amounts of foreign material, probably from the gelatin, in the bottoms of the tubes. These deposits were reddish brown before incubation, then became blackened as incubation progressed. The turbidity in the 20.0 per cent gelatin medium cleared more rapidly at room temperature than in the concentrations ranging from 10.0 to 14.0 per cent. The cause of the more rapid clearing is not known, but may be the smaller amount of precipitate formed in this concentration of gelatin. Prolonged incubation of the other species did not change the results recorded for them after 72 hours' incubation at 37° C.

Judging from these observations, the optimum concentration of gelatin for obtaining a maximum gallinarum reaction or a clear-cut negative test is between 10.0 and 15.0 per cent when 0.15 per cent cysteine hydrochloride is used.

EFFECT OF VARYING THE HYDROGEN-ION CONCENTRATION OF THE MEDIUM

To determine whether the hydrogen-ion concentration of the medium has a bearing on the gallinarum reaction, seven strains each of *Salmonella gallinarum* and *S. pullorum* were inoculated in tubes of 0.15 per cent cysteine hydrochloride gelatin medium which had been adjusted to H-ion concentrations varying from pH 5.6 to 7.6. Observations were recorded after 24, 48, and 72 hours' incubation at 37° C and again after an additional incubation of 36 days at room temperature.

The findings show a definite relation between the pH of the medium

and the characteristic reaction in the *Salmonella gallinarum* cultures, but little, if any, in the *S. pullorum* tests. The optimum pH for *S. gallinarum* appears to be 7.2 to 7.6 with a decreasing efficiency in the lower range. After prolonged incubation, no marked differences due to change in pH were noted, although a few strains remained cloudy, with the greatest turbidity at pH 7.6.

EFFECT OF INCUBATION TEMPERATURE

The relation between the incubation temperature and the gallinarum reaction has been studied in both tubed and plated media.

Incubation at 17° C.—Tubed medium inoculated by the puncture method while the gelatin was solid was incubated at 17° C. At this temperature *Salmonella gallinarum* (five strains) produced a fair growth along the line of inoculation. After 48 hours a cloudy zone appeared along this line. At about 1 mm below the surface and extending 4–5 mm into the medium, this zone was somewhat larger in diameter than on the surface or at points deeper in the medium. With continued holding, the cloudy zone increased in intensity and slightly in diameter, until after 120 hours it formed a band across the full width of the tube 1–2 mm below the surface. The cloudiness along the line of stab seldom extended deeper than half the length of the gelatin column, and increased in diameter towards the surface. Except for the surface zone and the zone along the upper half of the stab, the medium remained clear. The reaction cleared after prolonged incubation, the time required varying with individual strains, but being usually less than one month.

Four strains of *Salmonella pullorum* inoculated in the same lot of medium and incubated in the same low-temperature incubator with the *S. gallinarum* strains, never showed turbidity along the line of stab, though the growth of the organism was plainly visible. Plain nutrient gelatin tubes inoculated with one or the other of these two species never showed cloudiness; both species grew somewhat better in the medium containing cysteine.

Shake cultures of the same organism grown at 17° C developed turbid zones around each individual colony in all *Salmonella gallinarum* strains. In these instances the colony itself was plainly visible within the spherical turbid zone. *S. pullorum* produced no turbidity around the colonies of any strain studied.

Incubation at 20° C.—Five strains of *Salmonella gallinarum* were seeded into cysteine gelatin medium by both the puncture and the shake-dilution methods, with tubes containing 12.5, 15.0, and 20.0 per cent gelatin. Final observations were made after 9 days' incubation at 20° C. The results resembled those reported for the incubation trials at 17°. No

difference could be noted in the various concentrations of gelatin. The individual colonies in the more dilute shake cultures were round and well scattered throughout the medium. In general, those nearest the surface showed no turbid halo, nor did those below 1½ inches from the surface. Colonies in the areas between showed distinct cloudy halos and therefore appeared somewhat larger than in the areas where no reaction occurred. A zone of subsurface turbidity about 2–3 mm deep developed in part of the tubes.

RELATION OF CULTURE METHODS TO THE REACTION

To determine whether the type of culture container or the amount of medium placed in it has any bearing on the reaction obtained, several kinds of containers were used: test tubes, flasks of various sizes, and petri dishes. Different amounts of medium were employed.

Four different sizes of test tubes were used: 12-, 15-, 18-, and 22-mm. Medium to the depths of from 1 to 4 inches (in the 22-mm tubes) was inoculated with both *Salmonella gallinarum* and *S. pullorum*. After aerobic incubation at 37° C, no marked differences were noted in the efficiency of the medium in any of these combinations. In no case did *S. pullorum* produce turbidity. The only difference in *S. gallinarum* cultures was in the 22-mm tubes, filled to depths of from 3 to 4 inches. In these, less precipitate appeared than in the others, and the turbidity did not extend to a depth of over 2 inches unless the tubes were shaken. No method of measuring this reaction quantitatively was found. Judging from the above observations, 12- or 15-mm tubes containing 1½ to 2 inches of medium provide the most satisfactory procedures for routine studies.

Flasks of the Erlenmeyer type with various bottom diameters were tried to determine whether diameter and depth of medium are related to the amount of precipitate. Medium from 1 to 2 inches deep inoculated with *Salmonella gallinarum* and incubated at 37° C for 72 hours showed marked turbidity. When the reactions in flasks were compared qualitatively with the reactions in 22-mm tubes containing the same amount of medium, there appeared to be more precipitate in the flasks.

Three strains of *Salmonella gallinarum* and two of *S. pullorum* were transferred from 24-hour-old broth cultures to 15 cc of cysteine hydrochloride gelatin in amounts varying from 0.05 cc to 0.3 cc, and the seeded medium was poured into sterile petri dishes (100 mm × 15 mm). The plates were incubated aerobically at 22° C, and observed daily for two weeks. Good growth of both surface and subsurface colonies was noted, but in no instance was there turbidity. This method of inoculation and incubation was repeated with various lots of media, with similar results.

The findings conform to those obtained when *S. gallinarum* is grown in tubes of the same medium; there is no turbidity at the surface of the stab, and seldom is there any to a depth of 2–3 mm when the tubes are incubated at 20°–22° C.

VALUE OF OTHER MEDIA CONTAINING CYSTEINE HYDROCHLORIDE

The following experiments were conducted to determine whether a gallinarum reaction could be obtained when media other than nutrient gelatin are used as vehicles for cysteine hydrochloride.

Nutrient Agar.—The basal nutrient agar in these studies was prepared by the standard procedure. The only change was to vary the amount of agar as indicated in the trials summarized below.

Two agar media were used, one containing 0.5, the other 2.0 per cent agar. Twelve strains of *Salmonella gallinarum,* fourteen of *S. pullorum,* and five of *S. typhi* were inoculated into these media by the deep-stab method and the cultures were incubated at 37° C for 72 hours.

In the *Salmonella gallinarum* cultures a slight browning to a faint blackening occurred along the line of growth in both media with slightly better growth in the semisolid medium. No changes along the line of growth or in the surrounding medium were noted in either the *S. pullorum* or in *S. typhi* cultures. Slightly more intense blackening along the lines of growth was noted in the *S. gallinarum* cultures after 120 hours' incubation than after 72 hours' incubation. No changes were observed in the other species.

The reactions with the *Salmonella gallinarum* cultures cannot be considered striking enough to warrant using either of the two agar media for differential purposes. Pacheco and Mello (1932) used a bismuth carbonate agar in distinguishing between these organisms; and Pacheco and Rodrigues (1936*a*) obtained better results by adding 0.01 per cent cystine to the medium. These media were not used in the present studies.

Plain Meat-Extract Broth.—Nutrient meat-extract broth, prepared by the standard procedure for water analysis, was used as a basic medium. To this was added 0.15 per cent cysteine hydrochloride and the medium was adjusted to pH 7.2. At the time of the tests made with this medium, the same strains of organisms were inoculated into 0.15 per cent cysteine gelatin medium and into the standard meat-extract broth for control purposes. Approximately 8 cc of medium were introduced into each tube.

Sixteen strains of *Salmonella gallinarum* and seventeen strains of *S. pullorum* were inoculated into the three media mentioned above. All cultures were incubated for 72 hours at 37° C. No more turbidity was

noted in the broth tubes containing cysteine hydrochloride than in the control tubes. Because of the turbidity caused by growth, casual observation does not reveal any reaction that may occur. For this reason alone a broth medium is valueless as a vehicle for the cysteine hydrochloride.

Methylene Blue Broth.—To determine whether methylene blue may be useful as an indicator in cysteine hydrochloride broth, a medium containing 1:200,000 methylene blue was tubed in 8-cc quantities.

Fourteen strains each of *Salmonella gallinarum* and *S. pullorum* were inoculated into this medium. Observations were made after 24, 48, and 72 hours' incubation at 37° C. Except at the surface, the methylene blue was definitely reduced in all the *S. gallinarum* cultures in 24 hours. At the end of 48 hours there was complete reduction, but by 72 hours reoxidation had occurred. At the end of 120 hours, the *S. gallinarum* cultures were grassy green with a heavy deposit of white amorphous powder in the bottom of the tubes. Very slight reduction was noted in the *S. pullorum* cultures in 24, 48, or 72 hours; but at no time was the reduction as marked as in the case of *S. gallinarum* cultures. At the end of one week, no further changes were noted in either species.

Judging from these studies, methylene blue may serve as an indicator for the cysteine reaction in differentiating *Salmonella pullorum* from *S. gallinarum* in broth cultures.

Ice-Cream Gelatin.—A high-grade ice-cream gelatin was used in these studies. To determine whether this cheaper product could be employed as a basal medium for cysteine hydrochloride, it was compared with the regular cysteine gelatin. Twelve strains of *Salmonella pullorum*, fourteen of *S. gallinarum*, and five of *S. typhi* were each inoculated into the two cysteine media and into the same media without cysteine. The *S. gallinarum* reactions observed in the ice-cream gelatin medium differed only slightly from those in the regular product, but the natural cloudiness of the medium definitely interfered with the interpretation of the results. No changes were noted in either of the plain gelatin media, although that made from the ice-cream gelatin had the definite cloudiness noted above. As far as could be determined, the organisms grew equally well in all these media. Judging from these studies, the brand of ice-cream gelatin used could not be substituted for the Difco granulated gelatin of the regular medium.

Eastman De-ashed Gelatin.—This highly purified gelatin (isoelectric point of pH 4.7) is washed free of all soluble chemicals and supposedly has a very low sulfur content. It was used in a cysteine medium in place of Difco-Bacto gelatin for comparison with the latter medium. Owing to the cost of the Eastman product, both media were tubed in 2.5-cc quantities in 12-mm tubes.

Five strains each of *Salmonella pullorum* and *S. gallinarum,* four of *S. typhi,* three of *S. typhimurium,* and three of *S. bareilly* were seeded into the two media and incubated for 72 hours at 37° C. The Difco-Bacto gelatin medium apparently gave somewhat more pronounced reactions in 48 hours; but after 72 hours no differences could be noted.

There was seemingly no advantage in using the Eastman de-ashed product for routine purposes. Since the typical reaction is obtained with it, it may be used for chemical studies on the nature of the reaction in order to prevent sulfur (other than that found in the cysteine) from interfering with the results.

Scott's Synthetic Medium.—Since Scott's (1930) synthetic medium grows the *Salmonella* species satisfactorily, it was selected as a sulfur-free vehicle for gelatin and cysteine. The original formula calls for ferric citrate as one of the salts. In preliminary trials certain organisms of the *Salmonella* genus blackened the medium in the presence of gelatin and cysteine. For these studies both the original medium and one in which ammonium citrate was substituted for ferric citrate was used. The regular nutrient gelatin cysteine medium served as a control. All three were tubed in 2.5-cc quantities in 12-mm tubes.

Five strains each of *Salmonella pullorum* and *S. gallinarum,* four of *S. typhi,* three of *S. typhimurium,* and three of *S. bareilly* were inoculated into these media and incubated for 72 hours at 37° C. Before inoculation the medium containing the ferric citrate was dark brown, with the lower one third almost black. *S. pullorum* caused this blackening to disappear within 24 hours; no reaction or precipitate was observed. *S. gallinarum* caused a temporary increase in the blackening, followed within 48 hours by a deposit of a black flocculent precipitate in the bottom of the tubes. Very little cloudiness of the medium was noted. *S. typhi* reacted in a manner similar to *S. pullorum* in the ferric citrate medium. Two of the three *S. typhimurium* strains caused this medium to become coal black in 24 hours and retained this color for at least 96 hours; the other strain caused a reaction closely resembling that of *S. gallinarum,* with no permanent blackening of the medium. Of the three, this strain was the only one that gave a positive reaction in the control. All three strains of *S. bareilly* caused permanent blackening in 24 hours.

The ammonium citrate modification of Scott's medium produced good growth of all the organisms studied, and gave results identical with the regular cysteine gelatin medium for *Salmonella pullorum, S. gallinarum,* and *S. typhi.* The results with *S. bareilly* and *S. typhimurium* in this medium were variable. All the *S. bareilly* strains were negative in the control (regular cysteine gelatin) medium, while only one failed to give any reaction in the ammonium citrate. The latter medium was slightly

darkened by all of the *S. bareilly* cultures. Two of the *S. typhimurium* cultures were negative in both the control medium and the ammonium citrate. The third was positive in both media. Slight darkening of the ammonium citrate medium occurred in all of the *S. bareilly* and *S. typhimurium* cultures.

Time has not permitted a further study of these media; but judging from these preliminary results, Scott's medium and the ammonium citrate modification may prove valuable as vehicles for cysteine and gelatin in differentiating various *Salmonella* species.

COMPARISON OF CYSTEINE WITH RELATED COMPOUNDS

Since the reaction obtained when *Salmonella gallinarum* is grown in gelatin containing cysteine hydrochloride is apparently related to the sulfur contained in the cysteine, it was thought desirable to determine whether other sulfur-containing organic compounds would react similarly. The substances selected for this study were cystine, methionine, and glutathione.

Cystine and Methionine.—Cystine is an amino acid having an –S–S– group; it is readily reduced to cysteine. Methionine has the single –S– radical combination. To compare the effect of growing various *Salmonella* species in gelatin media containing these amino acids, amounts with approximately equivalent proportions of sulfur were added to the basic nutrient gelatin medium, and the medium was adjusted to a pH of 7.2. Two lots of gelatin media containing 0.15 and 0.12 per cent cystine and two lots containing 0.15 per cent methionine were compared with a lot of 0.15 per cent cysteine hydrochloride gelatin.

As the cystine would not go into complete solution, this medium had a milky opacity about equivalent to a partial gallinarum reaction. This fact alone would make cystine an undesirable substitute for cysteine hydrochloride.

Four strains of *Salmonella gallinarum,* six of *S. pullorum,* three of *S. typhi,* and eight miscellaneous *Salmonella* species were used for these comparative studies. After amounts of inoculum as nearly equivalent as possible had been introduced into the different tubes of medium, the cultures were incubated for 72 hours at 37° C. An additional day's incubation failed to change the results.

The reactions in the medium containing cystine resembled those in the one with cysteine hydrochloride. Methionine, however, caused no visible changes, except perhaps a slight increase in turbidity attributable to the growth of the organism.

Turbidity definitely increased in all *Salmonella gallinarum* cultures

containing cystine; and after 72 hours the reactions were similar to those observed in the cysteine gelatin cultures. In all *S. pullorum* and *S. typhi*-tubes and in a few of the miscellaneous *Salmonella* cultures containing cystine, the medium definitely cleared after 48 hours', in some instances after 24 hours', incubation. In some tubes this clearing was only partial; in others, complete. In a few this procedure was reversed; that is, when complete clearing had been noted, a cloudiness developed after an additional 24 hours' incubation. Whether these changes result from utilization of the finely suspended particles of cystine by the organism, or from reduction of the cystine to the more soluble cysteine, has not been deter-

TABLE 4

Hydrogen-ion Concentration of Cultures after Ten Days' Incubation

Cultures	pH of various media				
	Cysteine, 0.15 per cent	Cystine, 0.15 per cent	Cystine, 0.12 per cent	Methionine, 0.15 per cent	Methionine, 0.14 per cent
G1	6.8	6.8	6.6	7.2	7.4
G32	6.6	6.6	6.4	7.2	7.2
P15	7.2	7.0	7.2	7.4	7.2
P97	7.2	7.2	7.2	7.4	7.4
T2	7.2	7.2	7.2	7.4	7.2
T12	7.2	7.2	7.2	7.2	7.2
Control	7.2	7.2	7.0	7.2	7.2

mined. The reappearence of turbidity in a few cultures may be caused by a reoxidation of cysteine to cystine.

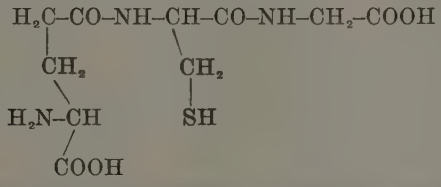
Judging from results, cystine may be used instead of cysteine to produce the gallinarum reaction; but its low degree of solubility makes it a poor substitute. Methionine cannot be considered a substitute for cysteine; the failure to produce the reaction probably means that the –SH radical of the cysteine is responsible for the reaction.

After 5 days' incubation at 37° C, half of the *Salmonella gallinarum, S. pullorum,* and *S. typhi* cultures were left at room temperatures for an additional 5 days. To each of the remaining cultures was added a drop of a saturated alcoholic solution of methylene blue. After a thorough shaking, observations were made for 1 hour. Except for one of the *S. typhi* strains tested, no differences could be noted in the reduction of the methylene blue. With this strain slight reduction occurred in the cysteine gelatin cultures, partial reduction in the cystine gelatin, and nearly complete reduction in the methionine gelatin. Only partial reduction of the methylene blue was noted in the *S. gallinarum* and *S. pullorum* cultures, with no differences in the several media.

The cultures which were kept over for 5 days at room temperature for

additional observations were subjected to pH determinations with the aid of bromthymol blue. The results appear in table 4. The control used in these studies consisted of an uninoculated tube of the medium referred to, incubated along with the inoculated tube.

Glutathione.—Glutathione was used as another example of an organic compound containing sulfur having the –SH group. The following formula from Bodansky (1938) was used for calculating the percentage of sulfur:



Glutathione (molecular weight 307.217) contains 10.44 per cent sulfur, as compared with 20.34 per cent in cysteine hydrochloride (molecular weight 157.587). To obtain a medium with the same amount of sulfur as in the 0.15 per cent cysteine hydrochloride gelatin, 0.29 gram of glutathione was added to 100 cc of nutrient gelatin. A medium containing 0.145 gram per 100 cc was also made. Two lots of cysteine hydrochloride gelatin, one containing 0.15 per cent and the other 0.075 per cent cysteine hydrochloride, served as controls. All of these media were tubed in 2.5-cc amounts in 12-mm tubes.

Five strains each of *Salmonella pullorum* and *S. gallinarum* were inoculated into the four media and incubated at 37° C. Observations were made at 24-hour intervals for 96 hours. Typical reactions occurred in all of the *S. gallinarum* cultures containing cysteine medium; on the other hand, glutathione medium remained clear as did all of the tubes inoculated with *S. pullorum*.

Judging from the results obtained with glutathione, the –SH group in cysteine is not entirely responsible for the gallinarum reaction. Possibly, however, *Salmonella gallinarum* may dissimilate cysteine somewhat differently from the manner in which it does glutathione, for the *S. gallinarum* cultures changed the cysteine medium slightly to the acid range of bromthymol blue, whereas there was a slight alkaline change in the glutathione medium. Growth was verified by streaking part of the cultures on agar after 96 hours' incubation. Because of the expense of glutathione, these studies were not repeated.

DISCUSSION

Classification of bacteria by means of fermentation reactions have until recent years been largely based on differences in fermentations of carbohydrates and alcohols by the organisms. The utilization of organic compounds, the production of H_2S, and the reaction towards various types of media are more recent and, in many respects, more reliable means of differentiation. This is especially true where organisms vary in their ability to attack certain carbohydrates. A good example is the variation in maltose-fermenting ability of *Salmonella pullorum*, a property once considered the most marked difference between this bacterium and *S. gallinarum*. Largely because of the variable reaction of *S. pullorum* toward maltose and because of antigenic likenesses, many investigators such as Miessner (1931), Wagener (1934), and Haupt (1935) regarded the two as variants of the same species. Although many variants do exist, there is increasing evidence that *S. gallinarum* and *S. pullorum* are distinct species. The differences in the reaction of these organisms toward the Jordan-Harmon (1928) tartrate agar (Mallman, 1931*a*; Hinshaw and Rettger, 1936), with the separation into distinct species by bacteriophage by Naidu (1935) and Nobrega (1935), exemplify recent methods for differentiating these species. The production of a characteristic reaction in cysteine gelatin by *S. gallinarum*, but not by *S. pullorum* as reported in this investigation, is added evidence that they are separate species. Whereas all but two of the *S. gallinarum* strains (G87 and G89) of avian origin produced the characteristic reaction, all 454 strains of *S. pullorum* were negative in this medium. All these strains also reacted typically for the species in tartrate agar medium.

Judging from the observations made in these studies, and those of Van Roekel (1935, 1937), Nobrega (1936), Kujiumgieff (1937), Barboni (1937), Pacheco and Rodrigues (1935*a*), Delpy and Rastegar (1938), there are probably several subspecies in the pullorum-gallinarum group. In table 5 are listed several strains of variants of *Salmonella pullorum* and *S. gallinarum*. These strains, some of which have been isolated in California and the others obtained from various laboratories, have been studied by the use of the more common differential media including cysteine gelatin and Jordan-Harmon tartrate agar. The results are summarized in the table. It will be noted that Van Roekel's (1935, 1937) maltose-fermenting strain (P52) gives identical reactions to the California maltose-fermenting strains of this organism. As found in the present studies, these strains have consistently fermented maltose within 72 hours, and generally within 24 hours, at 37° C. Several of the Cali-

fornia strains have been isolated from chicks or turkey poults that have died during an acute outbreak of pullorum disease, and fermented maltose on original isolation. P52 is designated as Massachusetts VII in Van Roekel's collection. The Van Roekel and California strains differ from the *Salmonella intermedius* A-type strains in that the latter are xylose-negative and dulcite-positive. These strains furnished by Nobrega and by Nai, are classified as *S. intermedius* A type according to Pacheco

TABLE 5

SUMMARY OF CHARACTERISTICS OF VARIANTS OF *Salmonella pullorum* AND *S. gallinarum*

Variants and stock numbers	Gas forming	Fermentation reactions in the media given*					
		Maltose	Xylose	Dulcite	Arabinose	Cysteine gelatin	Tartrate agar†
S. pullorum (Van Roekel strain); P52	+	+	+	−	+	−	−
S. pullorum (California strains); P75, P86–P89, P121, P184–P187, P222–P228, P230	+	+	+	−	± to +	−	−
S. intermedius A types; P130, P214, P217, P218	+	± to +	−	+	+	−	−
S. intermedius B types; P127–P129	−	+	−	+	+	−	−
S. gallinarum (Kujiumgieff strain); G87	−	+	−	−	+	−	+
S. gallinarum (Barboni strain); G89	−	−	+	+	−	−	+
S. gallinarum (Van Roekel strain); G84	−	−	+	+	+	+	+
S. gallinarum var. *duisburg*; G55–57, G85	−	+	±	+	+	−	−

* Explanation of symbols: + = positive reaction; ± = variable or slow fermentation; − = negative reaction.

† Jordan-Harmon (1928) tartrate agar

and Rodrigues (1935). In these studies Nai's strains (P214, P217, P218) did not ferment maltose except after several days' incubation. P214 is described as Bulgaria 7 by Kujiumgieff (1937) according to Nai. P217 and P218 are described by Barboni (1937) as Torino 15, and Brescia 21, respectively.

The *Salmonella intermedius* B types were also furnished by Nobrega (1935, 1936). They are classified by him as P7 (Rotterdam), P14 (Rotterdam), and P24 (Berlin). Nobrega found these strains, as well as P130, sensitive to *S. pullorum* bacteriophage and because of this fact considers the strains variants of *S. pullorum*. It will be noted that in these studies *S. intermedius* A and B types reacted like *S. pullorum* in both cysteine gelatin and tartrate agar. The writer has not found any American

strains of *S. pullorum* which conform with Pacheco and Rodrigues (1935) qualifications for *S. intermedius* A or B types.

Van Roekel's maltose-negative strain of *Salmonella gallinarum* (G84) was classified by him as *S. gallinarum* on the ground that it produced a positive gallinarum reaction in cysteine gelatin; acid in tartrate agar; and was dulcitol-positive. This strain was isolated from a chick that died in an acute outbreak of a disease resembling pullorum disease.

The dulcitol-negative *Salmonella gallinarum* variant (G87) of Kujiumgieff (1937) differs from Delpy and Rastegar's (1938) *S. intermedius* type B, in that the latter is dulcitol-positive and tartrate-negative. No attempt has been made to derive dulcitol-positive substrains from it. Barboni's (1937) maltose-negative strain (G89) and Kujiumgieff's (1937) strain (G87) are the only strains in either the *S. pullorum* or *S. gallinarum* collection which produced no reaction in cysteine gelatin and a positive reaction in tartrate agar. They are variants which are difficult to classify.

The Duisburg strains of *Salmonella gallinarum* have been discussed in another section. They are like *S. pullorum* in that they do not ferment tartrate agar with acid production, and do not give any reaction in cysteine hydrochloride gelatin. They resemble *S. gallinarum* in the type of growth on nutrient agar, and in being maltose- and dulcitol-positive. They are more nearly like the *S. intermedius* type B than any of the other strains given in table 5.

Barboni (1937), who has studied 63 strains belonging to the pullorum-gallinarum group, suggests a reclassification based on fermentation of d–tartrate, which is in the Jordan-Harmon (1928) medium. He would designate all positive fermenters (acid) as *S. gallinarum*, and all strains that do not attack the d-tartrate as *S. pullorum*. With gas production as a basis, he would reclassify the two species into non-gas- and gas-forming subspecies, as determined by neutral red fluorescence tests. Even with this regrouping, he has set apart an additional one which he calls *S. gallinarum intermedius*, largely on the basis of colony formation. This group of biochemical variants of both *S. pullorum* and *S. gallinarum* must undergo fundamental study before being classified as distinct species.

The cysteine gallinarum reaction here reported is a new type of differential reaction, which must be studied further before one can predict its physical or chemical nature. According to Buchanan and Fulmer (1930), quoting Czapek (1920), the following reactions caused by dissimilation of cysteine by microörganisms are possible:

$$\underset{\text{Cysteine}}{HS{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH} + H_2O \longrightarrow \underset{\beta\text{-thiolactic acid}}{HS{\cdot}CH_2{\cdot}CHOH{\cdot}COOH} + NH_3$$

The *B*-thiolactic acid is decarboxylated and oxidized to thioglycollic acid as follows:

$$\underset{\text{B-thiolactic acid}}{HS\cdot CH_2\cdot CHOH\cdot COOH} + O_2 \longrightarrow \underset{\text{Thioglycollic acid}}{HS\cdot CH_2\cdot COOH} + CO_2 + H_2O$$

The thioglycollic acid is further oxidized to methyl mercaptan (CH_3SH).

Yaoi (1925) showed that H_2S, but never CH_3SH, is formed from thiolactic or thioglycollic acid. Further, he noted that mercaptan is produced from cystine only in the presence of a sugar. According to Almy and James (1926), no mercaptan is produced from cystine or peptones by *Proteus vulgaris, Salmonella aertrycke,* and *Escherichia coli.* Yaoi (1925), using Frankel's synthetic medium with l-cystine, found H_2S to be formed by *E. coli, Salmonella typhi* and *S. paratyphi* A, but not by *Shigella dysenteriae.*

In a culture of *Salmonella gallinarum* growing in cysteine-gelatin medium, H_2S can be demonstrated readily by its faint odor and by the use of strips of lead acetate paper hung in the tube or flask during incubation. No mercaptan odor has been detected. The turbidity, however, cannot be accounted for by the H_2S, since it remains after growth has ceased and after H_2S can no longer be detected with lead acetate paper. Only a faint browning of such paper has been noted in *S. pullorum* cultures grown in cysteine-gelatin medium.

The H-ion concentration of the cysteine-gelatin medium is lowered as much as 0.6 pH during the growth of *Salmonella gallinarum,* but is not changed during growth of *S. pullorum.* This observation suggests a possible colloidal precipitate with gelatin; but if such a precipitate were formed, one would expect the optimum reaction to occur at a pH near the isoelectric point of gelatin. In favor of actual dissimilation of the chemical is the fact that both *S. pullorum* and *S. gallinarum,* as well as other *Salmonella* species, produced an initial clearing of a cystine gelatin suspension, as indicated by the disappearance of the cystine crystals. This clearing in *S. gallinarum* cultures is followed by the production of the characteristic cysteine reaction.

Another possibility, which can be solved only by a thorough chemical study, is that *Salmonella pullorum* and other organisms which produce no visible change in cysteine gelatin may actually dissimilate the cysteine into some other soluble compound or compounds. No supporting evidence has been found. Scott's (1930) synthetic medium, in which gelatin was substituted for agar and to which 0.15 per cent cysteine hydrochloride was added, was blackened by *S. bareilly* and by most strains of *S. typhimurium,* but was cleared by *S. pullorum. S. gallanarum,* on the other hand, produced a black flocculent precipitate,

which settled to the bottom of the tube after 48 hours. This medium, containing ferric citrate, on sterilization becomes brownish black. *S. pullorum* apparently utilizes the iron sulfide without evolution of any H_2S. This fact would suggest that the other species can dissimilate the cysteine more completely than does *S. pullorum*.

SUMMARY

Salmonella gallinarum in 89 out of 91 strains produced a characteristic yellowish-white or grayish turbidity when incubated at 37° C for 72 hours in a gelatin medium containing 0.15 per cent cysteine hydrochloride. After incubation at temperatures that do not liquefy the gelatin, a turbid halo appeared around the individual colonies in shake cultures, and along the line of inoculation in stab cultures. None of 19 other species of *Salmonella* studied gave this typical reaction. *S. paratyphi* A (2 strains) most nearly approached the gallinarum reaction, but it was not as clear-cut.

Species which consistently produced no visible change in the medium, or at best a surface pellicle, were *Salmonella pullorum* (454 strains), *S. gallinarum* variety *duisburg* (4 strains), *S. california* (7 strains), *S. cholerae suis* variety *kunzendorf* (11 strains), *S. derby* (3 strains), *S. dublin* (8 strains), *S. bredeney* (2 strains), *S. enteritidis* (1 strain), *S. give* (1 strain), *S. newington* (2 strains), *S. newbrunswick* (2 strains), and *S. paratyphi* B (2 strains). Species which were irregular in their reactions, but the majority of which gave negative results were *S. typhi* (14 strains), *S. anatum* (3 strains), *S. bareilly* (12 strains), *S. oranienburg* (2 strains), *S. typhimurium* (67 strains), and *S. typhimurium* variety *copenhagen* (15 strains).

Of 12 species from other genera, only two, *Pseudomonas aeroginosa* (4 strains) and *Proteus* from turkeys (5 strains), gave reactions resembling that produced by *Salmonella gallinarum*.

The optimum range of concentration of cysteine hydrochloride in gelatin for production of the gallinarum reaction is from 0.15 to 0.45 per cent. No advantage could, however, be seen in using concentrations greater than 0.15 per cent.

The optimum range of concentration of gelatin as a vehicle for cysteine hydrochloride is from 10 to 15 per cent. Concentrations above 15 per cent inhibit the reaction slightly; and concentrations below 10 per cent, though not affecting the typical reaction in *Salmonella gallinarum* cultures, produce false reactions with some organisms.

The optimum H-ion concentration for producing the gallinarum reaction is in the pH range of 7.2 to 7.6, with a decreasing efficiency as the pH is lowered beyond this.

The characteristic reaction was noted only when *Salmonella gallinarum* was grown in cysteine gelatin medium. Similar concentrations of cysteine hydrochloride in nutrient agar of various concentrations of agar, or in nutrient broth, were not characteristic enough to warrant their use.

The gallinarum reaction as shown by *Salmonella gallinarum* was produced when cystine was added to nutrient gelatin in quantities containing amounts of sulfur equivalent to that found in cysteine. Methionine and glutathione, however, were useless as supplementary compounds.

The Jordan-Harmon (1928) sodium potassium tartrate agar was a valuable supplementary medium to be used with cysteine gelatin for differentiating *Salmonella gallinarum* from *S. pullorum*. *S. gallinarum* (91 strains) consistently fermented this medium, with acid production, whereas *S. pullorum* (454 strains) produced no change in it.

Several maltose-fermenting variants of *Salmonella pullorum* have been observed in these studies. None of these has given a positive reaction either in the cysteine gelatin medium or in tartrate agar.

Two strains, diagnosed as *Salmonella gallinarum* by the donors because they are dulcitol- and tartrate-agar-positive, were studied. Both of these (G87 and G89) failed to produce a positive reaction in cysteine gelatin medium.

Pacheco and Rodrigues' (1935*a*) *Salmonella intermedius* B (4 strains) and Müller's *S. gallinarum* variety *duisburg* (4 strains), resemble *S. pullorum* in that they produce no change in cysteine gelatin, nor in tartrate-agar medium.

These studies furnish additional evidence that *Salmonella pullorum* (Rettger) and *S. gallinarum* (Klein) are separate and distinct species.

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LITERATURE CITED

Almy, L. H., and L. H. James.
1926. A method for the study of the formation of volatile sulfur compounds by bacteria. Jour. Bact. **12**:319–31.

Barboni, E.
1937. Contribute alla classificazione delle Salmonelle "Gallinarum-Pullorum." Clin. Vet. **60**:741–57.

Beach, J. R., and D. E. Davis.
1927. Acute infection of chicks and chronic infection of the ovaries of hens caused by the fowl-typhoid organism. Hilgardia **2**:411–24.

Beaudette, F. R.
1925. The possible transmission of fowl typhoid through the egg. Amer. Vet. Med. Assoc. Jour. **67**:741–45.
1930. Fowl typhoid and bacillary white diarrhea. 11th Internatl. Vet. Cong. (London) Proc. **3**:705–20.
1938. An outbreak of fowl typhoid in guineas. Amer. Vet. Med. Assoc. Jour. **92**: 695–98.

Beaudette, F. R., L. D. Bushnell, and L. F. Payne.
1923. Study of an organism resembling *Bacterium pullorum* from unabsorbed yolk of chicks "dead in the shell." Jour. Infect. Dis. **33**:331–37.

Beck, and R. Eber.
1927. Bakterielle weisse Ruhr der Kücken. Arch. f. Wiss. u. Prakt. Tierheilk. **56**:122–40.

Bergey, D. H., R. S. Breed, E. G. D. Murray, and A. P. Hitchens.
1939. Bergey's manual of determinative bacteriology. 5th ed. 1032 p. Williams and Wilkins Company, Baltimore.

Bodansky, M.
1938. Introduction to physiological chemistry. 4th ed. 686 p. John Wiley and Sons, New York, N. Y.

Brown, H. C., J. T. Duncan, and T. A. Henry.
1924. The fermentation of salts of organic acids as an aid to the differentiation of bacterial types. Jour. Hyg. [London] **23**:1–22.

Buchanan, R. E., and E. K. Fulmer.
1930. Physiology and biochemistry of bacteria. Vol. III. Effects of microorganisms on environment. Fermentative and other changes noted. 575 p. Williams and Wilkins Company, Baltimore.

Bunyea, H.
1927. An epizootic salpingitis of pullets with special reference to *Salmonella pullorum* infection. Amer. Vet. Med. Assoc. Jour. **70**:645–51.

Burnet, F. M.
1929. Relationships between heat-stable agglutinogens and sensitivity to bacteriophage in the *Salmonella* group. Brit. Jour. Exp. Path. **8**:121–29.

Bushnell, L. D., W. R. Hinshaw, and L. F. Payne.
1926. Bacillary white diarrhea in fowl. Kansas Agr. Exp. Sta. Tech. Bul. **21**:1–85.

Cruickshank, G. A.
1927. Employment of a double sugar medium for routine diagnosis of bacillary white diarrhea, fowl typhoid, and fowl cholera. Jour. Bact. **14**:435–39.

Czapek, F.
1920. Biochemie der Pflanzen. Vol. 3. Gustav Fisher, Jena, Germany.

DELPY, L., and R. RASTEGAR.
1938. Etude de souches americanis, asiatiques et européennes de microbes du groupe pullorum-gallinarum. Inst. Pasteur [Paris] Ann. **61**:536–64.

DUMARESQ, J. A.
1938. Comparative studies of *Salmonella pullorum* and *Salmonella gallinarum*. Australian Jour. Exp. Biol. and Med Sci. **16**:361–69.

EDINGTON, J. W.
1924. The bacteriological study of fowl typhoid and allied infections with special reference to three epidemics. Jour. Path. and Bact. **27**:427–37.

EDWARDS, P. R.
1928. The fermentation of maltose by *Bacterium pullorum*. Jour. Bact. **15**:235–44.

EDWARDS, P. R., D. W. BRUNER, and W. R. HINSHAW.
1940. A new *Salmonella* type isolated from turkeys: *Salmonella california*. Jour. Infect. Dis. **66**:127–29.

EDWARDS, P. R., and F. E. HULL.
1929. The transmission of bacillary white diarrhea among hens. Amer. Vet. Med. Assoc. Jour. **75**:333–36.

EMMEL, M. W.
1936. The etiology of fowl paralysis, leukemia, and allied conditions in animals. III. The intestinal flora of chickens affected with enteritis associated with intestinal parasitism. Florida Agr. Exp. Sta. Bul. **293**:1–17.

GAUGER, H. C.
1934. A chronic carrier of fowl typhoid with testicular focalization. Amer. Vet. Med. Assoc. Jour. **84**:248–51.
1937. A study of naturally infected cases of fowl typhoid. North Carolina Agr. Exp. Sta. Tech. Bul. **53**:1–63.

GOLDBERG, S. A.
1917. A study of the fermenting properties of *B. pullorum* (Rettger) and *B. sanguinarum* (Moore). Amer. Vet. Med. Assoc. Jour. **51**:203–7.

GRESSEL.
1928. Zur Differenzierung des *Bact. pullorum* (Rettger) vom *Bact. paradysenteriae*. (*Bac. gallinarum* Klein, *Bact. sanguinarium* Moore, and *Bac. typhi gallinarum alcalifaciens* Pfeiler.) Deut. Tierärztl. Wchnschr. **36**:291–97.

GWATKIN, R.
1927. *Salmonella pullora* infections. Ontario Vet. Col. Rept. **1926**:39–48.

HADLEY, P. B., D. W. CALDWELL, M. W. ELKINS, and D. J. LAMBERT.
1917. Infections caused by *Bacterium pullorum* in adult fowls. Rhode Island Agr. Exp. Sta. Bul. **172**:1–40.

HAUPT, H.
1935. IV. Zur Systematik der Bakterien. Die für Mensch und Tier pathogenen gramnegatnen alkalibildenden Stabschenbakterien. Ergeb. Hyg. Bakt. Immunit. u. Exper. Ther. **17**:174–230.

HENDRICKSON, J. M.
1927. The differentiation of *Bacterium pullorum* (Rettger) and *Bacterium sanguinarum* (Moore). Amer. Vet. Med. Assoc. Jour. **70**:629–44.

HINSHAW, W. R., and L. F. RETTGER.
1936. Cysteine-gelatin as a differential medium for *Salmonella pullorum* and *Salmonella gallinarum*. Soc. Exp. Biol. and Med. Proc. **35**:44–46.

HINSHAW, W. R., and T. J. TAYLOR.
1933. A chronic carrier of fowl typhoid of turkeys. Amer. Vet. Med. Assoc. Jour. **82**:922-26.

Jones, F. S.
1913. An outbreak of an acute disease in fowls due to *Bacterium pullorum*. Jour. Med. Res. **27**:471–79.
Jordan, E. O., and P. H. Harmon.
1928. A new differential medium for the paratyphoid group. Jour. Infect. Dis. **42**:238–41.
Jordan, E. O., and R. Victorson.
1917. Differentiation of the paratyphoid-enteritidis group. II. Lead acetate agar. Jour. Infect. Dis. **21**:554–55.
Kauffmann, F.
1929. Das Vorkommen von Paratyphus Newport-Bacillen in Deutschland. Ztschr. f. Hyg. u. Infectionskrank. **110**:161–68.
1930. Die technik der Typenbestimmung in der Typhus-Paratyphus Gruppe. Centbl. Bakt. Abt. I. Orig. **119**:152–60.
1934. Untersuchungen über die Duisburger Gallinarum Stämme. Centbl. Bakt. Abt. I. Orig. **132**:337–42.
Kauffmann, F., and C. Mitsui.
1930. Vergleichende Untersuchungen in der Typhus-Paratyphus Gruppe. Ztschr. f. Hyg. u. Infektionskrank. **111**:749–72.
Kerr, W. R.
1930. Selective media for the cultivation of *Bacillus pullorum* and *B. sanguinarum*. Jour. Compar. Path. and Ther. **43**:77–85.
Klein, E.
1889. Ueber eine epidemische Krankheit der Hühner, verursacht durch einen *Bacillus—Bacillus gallinarum*. Centbl. Bakt. **5**:689–93.
Komarov, A.
1932. Fowl typhoid in baby chicks. Vet. Rec. **12**:1455–57.
Koser, S. A.
1923. Utilization of salts of organic acids by the colon-aerogenes group. Jour. Bact. **8**:493–520.
1924*a*. Correlation of citrate utilization by members of the colon-aerogenes group, with other differential characteristics and with habitat. Jour. Bact. **9**:59–77.
1924*b*. Is the ability to utilize citrate readily acquired or lost by the colon-aerogenes group? Jour. Infect. Dis. **35**:315–22.
1926. Differential tests and their relation to habitat of the colon-aerogenes group. Jour. Bact. **11**:409–16.
Krumwiede, C., and L. A. Kohn.
1917. Studies on the paratyphoid-enteritidis group. IV. The differentiation of the members of the paratyphoid-enteritidis group from *B. typhosus*, with special reference to anaerogenic strains and observations on the fermentative characteristics of the avian types. Jour. Med. Res. **36**:509–18.
Kujiumgieff, I.
1937. Gior. di Batteriol. e Immunol. **19**:364. (Original not seen.)
Lovell, R.
1932. The *Salmonella* group of bacteria. Bul. Hyg. [London]. **7**:405–15.
Mallmann, W. L.
1931*a*. Use of organic acids for differentiation of *Salmonella pullorum* and *Salmonella gallinarum*. Soc. Exp. Biol. and Med. Proc. **28**:501–2.
1931*b*. Studies on bacteriophage in relation to *Salmonella* and pullorum disease. Michigan Agr. Exp. Sta. Tech. Bul. **109**:1–15.

MALLMANN, W. L., F. THORP, and M. SEMMES.
1928. A medium for the isolation of *Salmonella pullorum* and other members of the paratyphoid group from avian tissues. Amer. Vet. Med. Assoc. Jour. **73**:825–38.

MALLMANN, W. L., and D. SNYDER.
1929. A medium for the differentiation of *Salmonella pullorum*, *Salmonella gallinarum*, *Pasteurella avicida*, and *Escherichia coli*. Jour. Infect. Dis. **44**:13–15.

MANNINGER, R.
1930. Hühnertyphus und bakterielle Kückenruhr. 11th Internatl. Vet. Cong. (London) Proc. **3**:724–40.

MARTINAGLIA, G.
1929. A note on *Salmonella gallinarum* infection of ten-day old chicks and adult turkeys. So. African Vet. Med. Assoc. Jour. **1**:35–36.

MIESSNER, H.
1930. Die Pullorum Infektion des Hühnes. (Die weisse Ruhr der Küken-Hühnertyphus.) Deut. Tierarztl. Wchnschr. **38**:517–20.
1931. Bacillary white diarrhea—fowl typhoid. 4th World's Poultry Cong. (London) Proc. **1930**:428–34.

MOORE, V. A.
1897. Infectious leukaemia in fowls—a bacterial disease frequently mistaken for fowl cholera. U. S. Dept. Agr. Bur. Anim. Indus. 12th and 13th Ann. Repts. **1895–96**:185–205.

MORLEY, L. C., and P. W. WETMORE.
1936. Discovery of the organism of ulcerative enteritis. No. Amer. Wildlife Conf. Proc. **1936**:471–72.

MÜLLER, R.
1933. Hühnerenteritis und atypische Gartnerbakterien bei Gastroenteritiden durch Eierspeisen. München. Med. Wchnschr. **80**:1771–72.

MULSOW, F. W.
1919*a*. The differentiation and distribution of the paratyphoid-enteritidis group. IV. Avian paratyphoid bacilli: A comparative study of *B. pullorum* and *B. sanguinarum*. Jour. Infect. Dis. **25**:135–62.
1919*b*. Observations on the avian paratyphoid bacilli. Amer. Jour. Pub. Health. **9**:508–11.

MUNNÉ, J. V.
1937. Au sujet de la differentiation de *Salmonella pullorum* et *S. sanguinarum*, au moyen d'un bacteriophage specifique. Soc. de Biol. [Paris] Compt. Rend. **126**:1228.

NAIDU, P. M. N.
1935. Essai de differentiation de *B. gallinarum* (Klein) et de *B. pullorum* (Rettger) au moyen de la bactériophagie. Acad. Vet. de France Bul. **8**:306–11.

NOBREGA, P.
1935. Differenciação entre "*S. pullorum*" e "*S. gallinarum*." Papel importante do bacteriophago. Arch. do Inst. Biol. **6**:71–84.

PACHECO, C., and T. MELLO.
1932. Novo methodo para pesquisa do hydrogenio sulfurado nas culturas bacterianas. São Paulo Univ. Faculd. de Med. Ann. **8**. (Quoted by Pacheco and Rodrigues, 1936*b*).

Pacheco, G., and C. Rodrigues.

1935*a*. Nouveau representant des bacteries du groupe pullorum-gallinarum. Morphologie des colonies du groupe. Soc. de Biol. [Paris] Compt. Rend. **118**: 905–7.

1935*b*. Biologie des bacteries du groupe pullorum-gallinarum. Action sur les milieaux au lait et sur le rouge neutre. Soc. de Biol. [Paris] Compt. Rend. **119**: 888–89.

1935*c*. Biologie du groupe pullorum-gallinarum—Fermentation des sucres. Soc. de Biol. [Paris] Compt. Rend. **119**:889–90.

1936*a*. Biologie du groupe pullorum-gallinarum. Caracterisation des types qui composent le groupe. Soc. de Biol. [Paris] Compt. Rend. **121**:590–92.

1936*b*. O groupo pullorum-gallinarum em provas bacteriologicas comparativas. Inst. Oswaldo Cruz. Mem. **31**:591–705.

Pfeiler, W., and A. Rehse.

1913. *Bacillus typhi gallinarum alcalifaciens* und die durch ihn verursachte Hühnerseuche. Inst. f. Landw. Bromberg, Mitt. **5**:306.

Plastridge, W. N., and L. F. Rettger.

1930. An epidemic disease of domestic fowl caused by a hitherto undescribed organism of the *Salmonella pullorum* type. Jour. Infect. Dis. **47**:334–39.

1934. Virulence of *Salmonella pullorum*. Jour. Infect. Dis. **54**:23–34.

Pyle, N. J.

1926. The bacteriophage in relation to *Salmonella pullora* infection in the domestic fowl. Jour. Bact. **12**:245–60.

Rettger, L. F.

1900. Fatal septicemia in young chicks. New York Med. Jour. **71**:803.

1901. Fatal septicemia in young chicks. New York Med. Jour. **73**:267.

Rettger, L. F., and S. A. Koser.

1917. A comparative study of *Bacterium pullorum* (Rettger) and *Bacterium sanguinarum* (Moore). Jour. Med. Res. **35**:443–58.

Rettger, L. F., and W. N. Plastridge.

1932. Pullorum disease of domestic fowl. Connecticut Agr. Exp. Sta. [Storrs] Bul. **178**:1–192.

Rodrigues, C., and G. Pacheco.

1936*a*. Biologie du groupe pullorum-gallinarum. Observations relatives a la differentiation des types pullorum par la fermentation de la maltose. Soc. de Biol. [Paris] Compt. Rend. **123**:438–40.

1936*b*. Biologie du groupe pullorum-gallinarum—relations and anti-geniques. Soc. de. Biol. [Paris] Compt. Rend. **123**:715–16.

Salmonella Subcommittee (Nomenclature Committee of the International Society of Microbiology).

1934. The genus *Salmonella*, Lignieres, 1900. Jour. Hyg. **34**:333–50.

Savage, W. G., and P. B. White.

1925*a*. An investigation of the *Salmonella* group with special reference to food poisoning. [Gt. Brit.] Med. Res. Council Spec. Rept. Ser. **91**:1–157.

1925*b*. Food poisoning—a study of 100 recent outbreaks. [Gt. Brit.] Med. Res. Council Spec. Rept. Ser. **92**:1–112.

Scott, J. P.

1930. A simple mineral agar for the cultivation of *Pasteurella boviseptica*. Jour. Bact. **20**:9–14.

SIMMONS, J. S.
1926. A culture medium for differentiating organisms of typhoid colon aerogenes groups and for isolating certain fungi. Jour. Infect. Dis. 39:209–14.
SMITH, T., and C. TENBROECK.
1915*a*. Agglutination affinities of a pathogenic bacillus from fowls (fowl typhoid) (*Bact. sanguinarum*, Moore) with the typhoid bacillus of man. Jour. Med. Res. 31:503–21.
1915*b*. A note on the relation between *B. pullorum* and *B. sanguinarum*. Jour. Med. Res. 31:547–55.
SPRAY, R. S., and L. P. DOYLE.
1921. Paratyphoid bacilli from chicks. Jour. Infect. Dis. 28:43–47.
STAFSETH, H. S.
1931. Acute pullorum disease in an adult hen. Amer. Vet. Med. Assoc. Jour. 79:818.
TITTSLER, R. P.
1930. Septicemic *Salmonella pullorum* infection in adult fowls. Poultry Sci. 10: 17–23.
1931. The effect of temperature upon the production of hydrogen sulfide by *Salmonella pullorum*. Jour. Bact. 21:111–18.
TITTSLER, R. P., and L. A. SANDHOLZER.
1935. The use of semi-solid agar for the detection of bacterial motility. Jour. Bact. 29:15.
TOPLEY, W. W. C., and G. S. WILSON.
1936. The principles of bacteriology and immunity. 2nd ed. 1645 p. William Wood and Co., Baltimore.
VALLEY, G.
1929. The favorable role of cysteine in the cultivation of anaerobic organisms. Jour. Bact. 17:12–13.
VAN ROEKEL, H.
1935. A study of variation of *Salmonella pullorum*. Massachusetts Agr. Exp. Sta. Bul. 319:1–60.
1937. Maltose-fermenting *S. pullorum* strains. Massachusetts Agr. Exp. Sta. Bul. 339:88.
WAGENER, K.
1934. Kückenruhr. 12th Internatl. Vet. Cong. [New York] Proc. 3:108–31.
WELDIN, J. C., and H. J. WEAVER.
1929. The production of gas by *Salmonella pullorum*. Rhode Island Agr. Exp. Sta. Bul. 222:1–25.
WELDIN, J. C., and A. R. MILLER.
1931. Utilization of citric acid and of sodium citrate by *Salmonella pullorum*. Rhode Island Agr. Exp. Sta. Bul. 232:1–16.
WHITE, P. B.
1926. Further studies on the *Salmonella* group. [Gt. Brit.] Med. Res. Council Spec. Rept. Ser. 103:1–160.
YAOI, H.
1925. On the sulphur-containing gas production by bacteria. Govt. Inst. Infec. Dis. Sci. Repts. (Japan) 4:129–38.

MICROANATOMY OF THE DUODENUM OF THE TURKEY

LAUREN E. ROSENBERG

MICROANATOMY OF THE DUODENUM OF THE TURKEY[1]

LAUREN E. ROSENBERG[2]

INTRODUCTION

THE PATHOLOGIST or physiologist dealing with mammalian subjects may refer to an extensive literature on normal histology when desiring to make comparisons with the phenomena he encounters. Workers in avian pathology or physiology find, on the other hand, that the histology of birds is not well known and that the articles are inconveniently scattered in a variety of journals in several languages. No comprehensive work on the complete normal histology of birds or of a single bird species has ever been published.

Recent attempts to solve problems dealing with intestinal disturbances in flocks of chickens and turkeys have emphasized this inadequacy. The present contribution deals with the detailed structure of the duodenum of the domestic turkey, *Meleagris gallopavo*.

To date, the best work on the histology of the bird intestine appears in eight papers by Clara (1925 to 1927*b*, inclusive),[3] constituting a comparative study of representatives of several orders of birds, including the Galliformes, but with nothing specifically on the turkey. Calhoun (1933) discusses the gross histology of the digestive tract of the domestic fowl, *Gallus gallus domesticus*, but her account is inadequate for the finer details of cell structure.

Though this paper deals mainly with tissues of the adult, it contains a brief section on the conditions obtaining in younger birds. The normal adults used were over eight months of age.[4]

GROSS STRUCTURE

The duodenum in the turkey is considered to be that part of the small intestine extending from the exit of the gizzard to the entrance of the pancreatic ducts. From the gizzard the duodenum makes a long posterior loop within which are the lobes of the pancreas, the whole being held together by a mesentery.

[1] Received for publication May 19, 1939.

[2] Instructor in Zoölogy, and Junior Zoölogist in the Experiment Station.

[3] See "Literature Cited" for complete data on citations, which are referred to in the text by author and date of publication.

[4] The turkeys used in this investigation were reared under routine conditions by the Division of Poultry Husbandry and showed no symptoms of disease or malnutrition. Thanks are due to Dr. V. S. Asmundson for his coöperation in this matter.

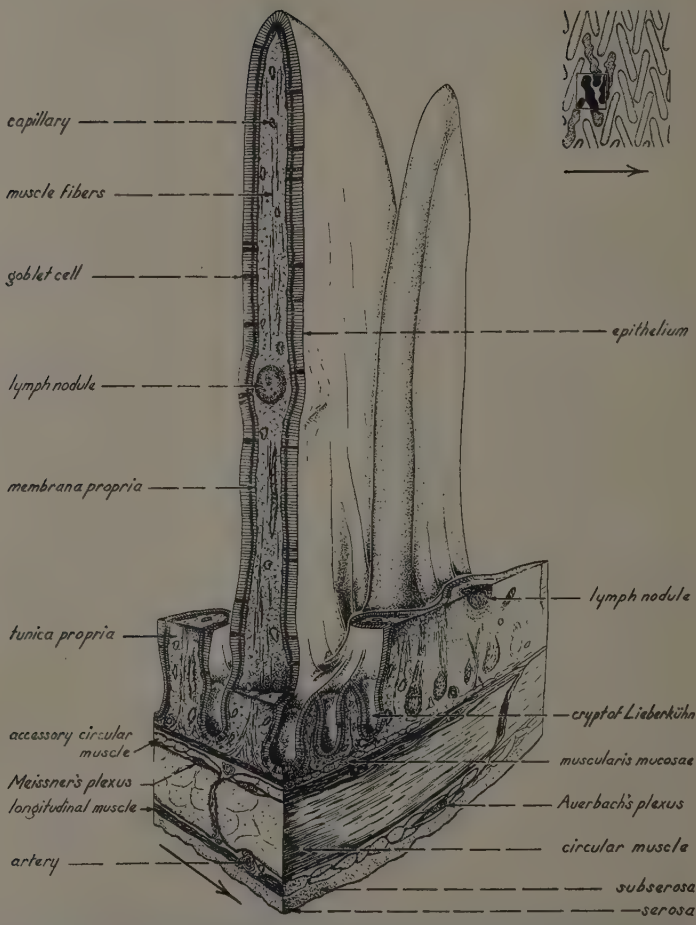


Fig. 1.—Idealized stereogrammatic section of turkey duodenum × 40. (The inset is a surface view of intestine with the blackened villi representing the area from which such a section might be taken.) The arrows indicate the longitudinal axis of the intestine.

In total length the duodenum varies from 29 to 39 cm; its outside diameter is roughly 10 mm; and the wall is approximately 3.5 to 4 mm thick.

When opened and washed free of intestinal mucus and debris, the internal surface of the duodenum presents a velvety appearance because of the long, close-set, tonguelike plates, the villi, that cover the entire surface. The villus plates are set in a precise geometric fashion, forming zigzag rows longitudinally and circularly (fig. 1; plate 1).

GENERAL MICROSCOPIC STRUCTURE

The villi are covered by a typical columnar epithelium continuous with that of the crypts. The Lieberkühn crypts are simple, unbranched, saclike structures, regularly placed and fairly constant in number. In two consecutive sections of duodenum, each 1 square millimeter in area, the author has counted 132 and 133, respectively. The cores of the villi and all available spaces around the crypts are occupied by the tunica propria, a loosely aggregated bed of connective tissue containing many free cells of various sorts, lymph nodules, blood vessels, and nerve fibers.

Brunner's glands are not present in the duodenum of the turkey. Clara did not mention them in any of the forms he studied. Calhoun (1933) does state that such glands are present in *Gallus gallus domesticus* for a short distance in the region between the gizzard and duodenum. Although Kaupp (1918) mentions Brunner's glands for *G. gallus domesticus,* his figure shows them above the muscularis mucosae.

In mammals a well-defined submucosa is present between the muscularis mucosae and the muscularis proper. In birds generally, a submucosa is not developed. In portions of the intestine where heavy folds are formed, a temporary submucosal area is indicated. The muscularis of the turkey is made up of three layers of muscles in contrast to mammalian forms, which show but two. The extra, or accessory, layer, lying adjacent to the muscularis mucosae, consists of circular muscle about as thick as the muscularis mucosae. Adjacent to this accessory layer is a second circular layer, comparable in thickness to that of mammals. In the turkey it is nearly twice as thick as the accessory layer and is composed of freely anastomosing bundles of fibers placed a little diagonally. Because of their oblique arrangement, one does not get a clear picture of entire cells in a cross section of the gut. A well-developed layer of longitudinal muscle bundles comprises the outer lamina of the muscularis. In thickness it is about the same as either the muscularis mucosae or the accessory circular layer. All the muscular coats of the intestine vary considerably in thickness, according to the degree of contraction or relaxation of the gut wall.

Between the muscularis and the serosa proper lies the subserosa, a layer of connective tissue. The serosa is a single-celled layer of peritoneum.

Nerve plexuses comparable to those of mammals are present. In mammals Meissner's plexus is located in the submucosa, and Auerbach's myenteric plexus is located in the muscularis between the longitudinal and circular layers (Maximow and Bloom, 1938). In the turkey, although both plexuses are found, they vary slightly in their position from the mammalian pattern. Both are located in the muscularis. Meissner's

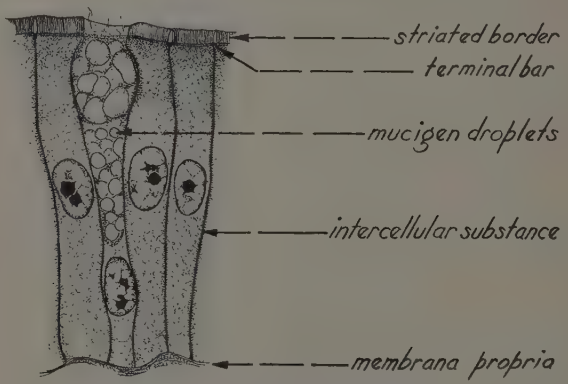


Fig. 2.—Goblet cell of villus epithelium × 1280 (compare plate 3, *E*).

plexus lies between the two layers of circular muscle and is connected by cross branches with Auerbach's plexus, which in the turkey lies in the connective tissue between the muscle bundles of the longitudinal layer.

DETAILED MICROSCOPIC STRUCTURE

The villi are long lamellae whose surfaces are covered with columnar cells of three types: (1) the chief cells, comprising the greater majority of them; (2) the goblet, or mucous, cells, also numerous; and (3) some basal granular cells.

Chief cells (figs. 1; 2; 3, *A;* plates 3, *C, D, E;* 5, *D*) are simple columnar cells that assume a hexagonal cross section because of the pressure of neighboring cells. In an adult bird they average 54 μ in height and 6 μ in width. The distal, or lumen, end of each is covered with a striated cuticular border. In the distal third of the cell is an oval nucleus containing one or two basophilic nucleoli and considerable dispersed chromatin. Acidophilic nucleoli have not been identified in

any of the author's material. The cytoplasm is acidophilic with some basophilic granules. On the lumen side of the nucleus there is a light-staining region proximally and a more basophilic area distally.

The goblet cells (figs. 1; 2; plate 3, *D, E*) resemble those of mammals. Their nuclei, though slightly smaller than those of the chief cells, are otherwise identical in morphology and staining capacity. The end of the cell toward the lumen does not possess a striated border like that found on chief cells. Goblet cells occur among the chief cells, but only rarely are two found side by side. In a small area the various goblet cells may be in different stages of activity. According to Clara (1926*a*), goblet cells of birds are extremely active; a second cycle of mucous secretion may be initiated before the first accumulation has been discharged.

Basal granular cells are found on the villi only in small numbers (fig. 3, *A;* plate 5, *D*). At their bases they are about the same in width as the chief cells, but the distal portion of each tapers and disappears near the lumen. The majority of the granules are located proximal to the nucleus and are decidedly basophilic, whereas in mammals they are acidophilic. The nuclei are smaller and more spherical than those of the chief cells. Although basal granular cells are easily demonstrated in turkey tissues by all the ordinary techniques, the most striking results are obtained with Susa-Heidenhain fixation, commonly called "Susa's fixation," followed by Regaud's hematoxylin (see table 1, p. 646).

In the literature the basal granular cells are mentioned under a variety of names, including *"gelben"* (Schmidt, 1905), *"gekörnten"* (Clara, 1925), *"chromaffinen"* and *"azidophil"* (Kull, 1913), *"argentaffin"* (Masson, 1928), *"Kultschitzky"* (Kultschitzky, 1897), and *"enterochromaffin"* (Ciaccio, 1907).

The cells of the villus epithelium rest upon a basement membrane, the membrana propria (figs. 1; 2; 3, *A;* plate 3, *C*), which separates the epithelium from the tunica propria. The membrana propria of the villus is continuous with that around the Lieberkühn crypt (figs. 1; 4, *A, B;* 6; plates 5, *A, B, C, E;* 6, *D*).

Mitosis has not been observed in any of these three cell types of the villi.

The crypts of Liberkühn (fig. 1) are situated at the bases of the villi, from which they arise singly or in groups. Their epithelium is a continuation of the villus epithelium, and the three cell types of the villus are likewise found in the crypts. The chief cells are shorter (20 to 22 μ in height), with a more basophilic cytoplasm. The striated border becomes less distinct toward the base of the crypt, but the terminal bars remain well developed. The goblet cells, though comparatively more

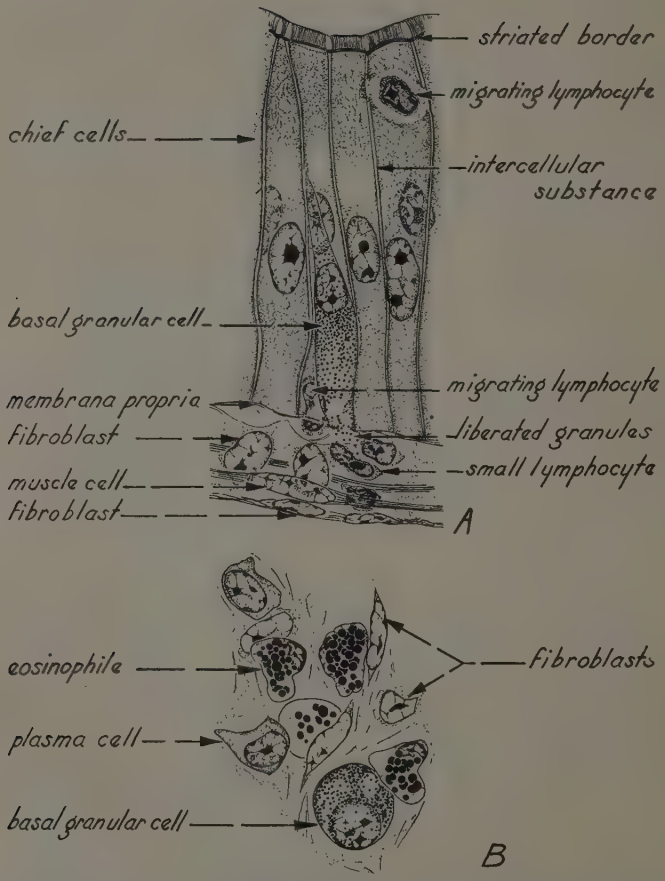


Fig. 3.—*A*, A portion of villus epithelium to show typical chief cells, a basal granular cell, and other neighboring elements × 1280. The basal granular cell is liberating some of its granules into the tunica propria. A lymphocyte is penetrating the membrana propria at the base of this cell (compare plate 5, *D*). *B*, A portion of tunica propria from the crypt region to show a free basal granular cell, eosinophiles, and neighboring elements × 1280 (compare plate 5, *E*).

numerous, are identical in structure with those of the villus mucosa. Basal granular cells are fairly numerous and are located principally in the deepest part of the crypt (fig. 4, *A, B;* plate 5, *A, B, C*), where they assume a more conical shape with the apex of the cone toward the lumen of the crypt. In such cases the distal pole of the cell is too small for a striated border; in fact, it is hard to demonstrate that the apex of the cell really reaches the lumen side of the epithelium. The nuclei are spherical, with well-stained dispersed chromatin and one or two nucleoli. The granules, small and numerous, staining intensely with chromatin stains and often obscuring a part of the nucleus, are mostly massed in the proximal part of the cell with the nucleus just distal to them. The nucleus has been likened to a ball valve (Macklin and Macklin, 1928) serving to hold the granules at the proximal pole of the cell. As shown by Masson (1914) for human beings, the basal granular cells occur singly, and rarely will two be found side by side. Occasionally one sees a few granules on the lumen side of the nucleus. Liberation of the granules into the lumen of the crypt has not previously been observed in any of the vertebrates studied, nor has the fate of the granules ever been determined. Cordier (1921, 1923) considers the basal granular cells to be exocrine in function, comparable with Paneth and pancreatic cells. Kull (1925), Parat (1924), and Masson and Berger (1923) believe them to be endocrine in function. According to Kull and Parat, the secretions of these cells are poured into the blood stream; but Masson calls them *"sympaticotropen"* glands, believing their products to be secreted into nerve endings in the mucosa.

The present writer has observed several instances, in stained sections, of the liberation of the granules; but in each case a migrating lymphocyte has been found penetrating the membrana propria at one side of the base of the basal granular cell. The liberated granules lie free in the tunica propria. The liberation has been seen in the cells of both the villi and the crypts (figs. 3, *A;* 4, *A;* plate 5, *B, C, D*).

Basal granular cells are not confined to the mucosa of the villi and crypts but occasionally occur free in the tunica propria (fig. 3, *B;* plate 5, *E*). In the latter event they are no longer conical but either spherical or oval, with the granules more evenly distributed instead of being confined to one end of the cell; and the nucleus is spherical and eccentric.

As Masson (1928) has shown, basal granular cells in man may proliferate to cause intestinal cancers, and may also migrate from the crypt into nerve tracts and cause nerve hyperplasias. According to him, all mitosis of these cells occurs in the epithelium of the crypt. The present writer, however, has not encountered recognizable cases of mitosis in these cells in the turkey.

In man the basal granular cells are found in the intestinal mucosa about the fourth month of fetal life and appear to be derived directly from the cells of the endoderm (Masson, 1928). From observations of

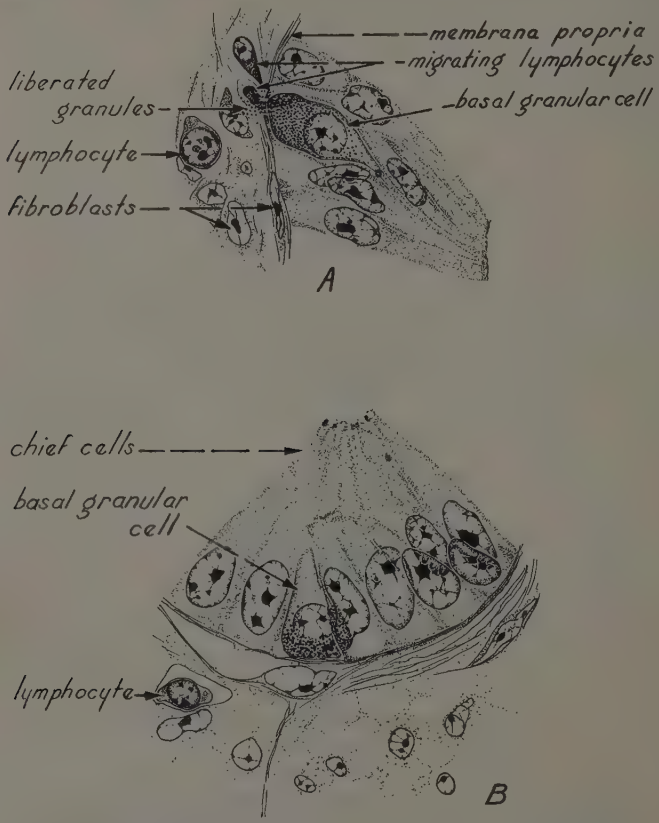


Fig.4.—Sections from the crypts of Lieberkühn × 1280. *A*, Liberation of granules from a basal granular cell with concurrent migration of a lymphocyte through the membrana propria (compare plate 5, *B*). *B*, A normal basal granular cell at the base of a crypt (compare plate 5, *A*).

the developing chick embryo, Kull (1913) concluded that they were formed from mesenchymal cells which later invade the epithelium. Simard and Campenhaut (1932), having restudied the development of basal granular cells in the chick, have reached conclusions similar to those of Masson for man. In the chick these cells do arise in the epithelium and

are thus of endodermal origin. They first appear around 264 hours of incubation, when a few are found at the very end of the anterior intestine, just above the umbilical pedicel. Further differentiations proceed caudally and cranially from this point, and by 336 hours of incubation the cells occur throughout the length of the intestine. By chorioallantoic grafts Simard and Campenhaut were able to show further that basal granular cells will differentiate in the absence of nervous elements. Those in the tunica propria are considered to be migrants from the epithelium. Although this phase of development has not been studied in the turkey, it is probably similar.

Numerous leucocytes are found between the epithelial cells of both the villus and the crypt; some are old lymphocytes which will eventually

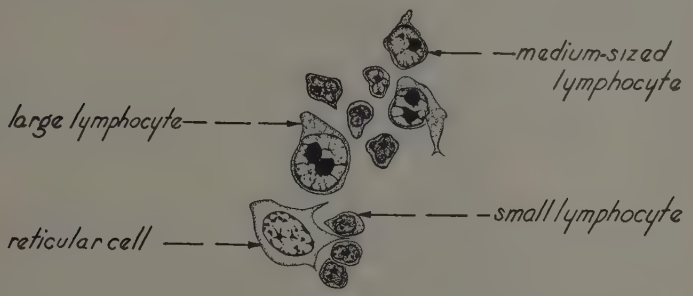


Fig. 5.—A portion of the tunica propria from the villus to show the relative features of the three types of lymphocytes × 1280 (compare plate 7, *C*).

reach the lumen of the intestine and be destroyed (figs. 3, *A;* 4, *A;* plates 3, *C, D;* 5, *B, C, D*). Others, less numerous, are the "*Schollenleukozyten*" of Weill (1919) (fig. 6; plate 6, *D*), of unknown origin; no definite function has been ascribed to them. Weill suggested that they may be secretory; and Keasbey (1923) agrees that they may be cells of external secretion comparable to pepsiniferous cells, although developmental stages are lacking. The schollenleucocytes are generally found in the proximal half of the cell layer and, although never seen to be extruded, are sometimes found near the crypt lumen. In the turkey they are irregular cells of varying sizes with oval to round, darkly staining nuclei. The cytoplasm is clear, with two to ten globules of varying size that are amphophilic after all methods of fixation. After the Susa's fixation, the schollenleucocytes and true eosinophiles appear to be different stages of the same cell, because of their staining affinities and the similarity of their nuclei. Since the granules of the eosinophiles do tend toward basophilic staining, the schollenleucocytes might well represent a phase in

the degeneration of the true eosinophiles. Since, however, the latter occasionally migrate through the epithelium without resembling schollenleucocytes, two distinct cell types may be indicated.

The crypt of Lieberkühn is the site of an enormous amount of mitosis from hatching to and throughout mature life (plate 4, *A*). As in mammals, mitosis is not found in the cells of the villus epithelium. Since shedding of old cells occurs on the villus, replacement must come from the crypt—a theory first postulated for mammals by Bizzozero (1893). In the turkey the theory is strikingly borne out. The cells rest lightly upon the membrana propria and are evidently capable of movement along

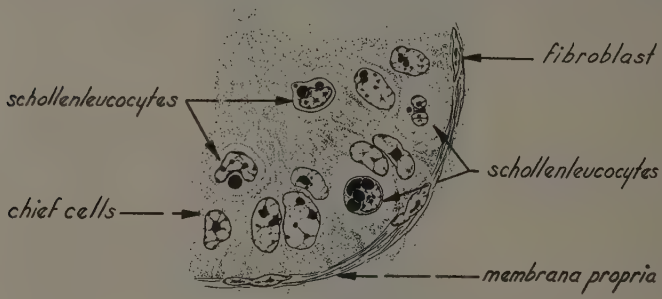


Fig. 6.—Schollenleucocytes in the epithelium of a crypt of Lieberkühn × 1280 (compare plate 6, *D*).

that membrane from crypt to villus tip. Mitosis in the crypt is far more frequent in the crypts of the duodenum than in the more posterior regions of the intestine. As shown by examinations of various levels of the small intestine, mitoses grow fewer from duodenum to rectum. The theory that crypt cells replace villus epithelium is further borne out by the ease with which the epithelial cells break away from the membrana propria when fixation is not accomplished with sufficient rapidity (Rosenberg, 1940).

Another type of cell often encountered in the Lieberkühn crypts of vertebrates is the Paneth cell, flask-shaped to columnar, with eosinophilic granules in the cytoplasm in the lumen side. This type is not found universally in any group of animals. Although Clara (1926*c*) describes Paneth cells for the Turdidae and for *Anas,* he failed to find them in other groups, including the Galliformes. The present writer agrees with this observation, for such cells have not been located in *Meleagris* by any of several techniques. Tang (1922) postulates a reciprocal relation between basal granular cells and Paneth cells: if one is present in large numbers, the other is lacking. This is the case in turkeys, for basal granu-

lar cells are numerous in almost any well-fixed 5-μ section. In the bird kingdom, according to Clara (1926*c*):

. . . . das Auftreten von derartigen Körnchenzellen in den Krypten verschiedenen Wirbeltiere ist ein Ausdruck für die weitgehende Differenzierung des Darmepithels, im besonderen des Kryptenepithels, was auch darin sich offenbart, dass bei den Vögeln nur in gut ausgebiedeten Krypten bisher solche Zellen gefunden werden könnten.

As *Meleagris* has a fairly simple type of Lieberkühn crypt, it would not be expected to possess Paneth cells.

The tunica propria of the adult turkey contains several cell types, and its character varies from the muscularis mucosae to the villus tip. The cores of some villi contain lymph nodules (fig. 1; plates 2, *B, C;* 7, *A, B*) whereas others lack them. In the sections studied, no nodule occurred within 600 μ of the villus tip. Often more than one nodule occurs in a villus, some being even as far down as the base of a crypt but always on the lumen side of the muscularis mucosae. Thus the lymph nodules in the turkey duodenum are not comparable with mammalian Peyer's patches since the latter are distinctly submucosal in position. Throughout the propria, fibroblasts are found, accompanied by strands of white collagenous connective tissue. Elastic fibers (yellow elastic connective tissue) are not present in the tunica propria.

The propria of the turkey is heavily permeated with lymphocytes of various types. Clara (1925) found the tunica propria of all the birds he studied to be dense with them—heaviest in the chicken and duck and least so in the pigeon. Lymphocyte formation and multiplication occur throughout the tunica propria, though the greatest activity is naturally in the lymph nodules. "In der Regel," remarks Clara, "können wir aber sagen stellt die Tunica propria ein retikuläres, zytogenes Gewebe dar, in dem stellenweise die faserigen Elemente überweigen. . . ."

The lymph nodules of the turkey are solitary, being equivalent to the secondary nodules of mammals. Within a nodule are reticular fibroblast cells, regularly distributed to form a suitable framework in which the nodular pulp cells may develop. The majority of cells within a nodule are lymphocytes of three distinguishable sizes: (1) Large lymphocytes (fig. 5; plate 7, *C*) are sparsely scattered throughout. They average 10×7.5 μ, and the nucleus has a diameter of 7 μ. The nucleus generally contains two nucleoli or a single bilobate one about 2.5 μ in diameter. The cytoplasm is basophilic, with some granulation. (2) Medium-sized lymphocytes (figs. 5; 7; 8; plates 7, *C, D, E*) are chiefly present in the medulla and differ from the preceding in size. They average 5×6 μ, and the nucleus has a diameter of 5 μ. The cytoplasm is the same as that of large lymphocytes. The medium-sized lymphocytes have usually a single nucleolus, occasionally two. (3) The small lymphocytes (figs. 3,

A; 5; 8; plates 5, *D;* 7, *C, E*), most abundant in the cortical regions, vary considerably in size. The nucleus may be as small as 2 μ in diameter, in which case it will stain darkly, or as large as 3.5 μ, when it is of a more typical wheel type. The latter, probably the younger form of the two, is presumably the ancestor of plasma cells and larger lymphocytes. The cytoplasm of small lymphocytes is also basophilic; it varies in amount, being sometimes so scant as to form only a slight border around the nucleus, and again it is abundant, in which event the nucleus is eccentric.

According to Jordan (1935) and Latta (1921), the various lymphocytes are not distinct forms but different growth stages of the same cell.

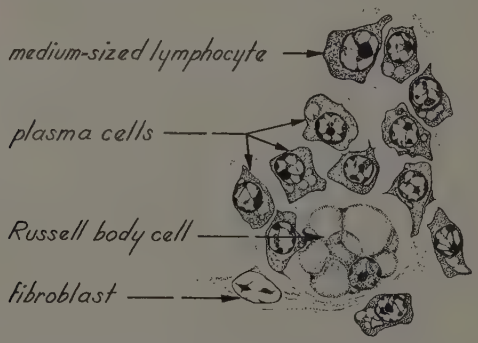


Fig. 7.—A portion of tunica propria from the lower part of a villus to show a Russell body cell and neighboring elements × 1280 (compare plate 7, *D*).

The large lymphocytes divide to form small ones, which in turn grow to medium size. In the turkey all three sizes of lymphocytes are also found in the tunica propria outside the nodule.

The most numerous cells of the tunica propria are the plasma cells (figs. 3, *B;* 7; plates 5, *E;* 7, *D*), generally conceded to be formed from small lymphocytes and incapable of further division. In structure, plasma cells resemble the young, small lymphocytes. They possess a typical wheel nucleus, with a well-defined central nucleolus, and the nucleus measures 3 to 4 μ. The cytoplasm is basophilic and possesses one to several vacuoles near the nucleus. The latter is generally eccentric in the cell body, which has a diameter of approximately 5 μ; but the cell shape varies from spherical to elongate.

Russell body cells (figs. 7; 8; plates 7, *D, E*) are another type encountered in the propria of vertebrates. Thought to be formed most commonly from plasma cells, they have been reported as abundant only in pathological conditions. In the normal turkey, one finds them only occa-

sionally; they vary in size from the dimensions of a plasma cell up to 12 μ in diameter, the latter thus being the largest cells occurring in the propria. Smaller ones may have a slightly eccentric, typical wheel nucleus. The cytoplasm is filled with numerous acidophilic globules, the Russell bodies. Large cells contain from two to many of these large acidophilic globules, and the nucleus may be pushed to the side of the cell and have its finer morphology obscured. Regarding the formation of Russell body cells, Maximow and Bloom (1938) state that when plasma cells degenerate, "large spherical drops or crystals of a peculiar acidophil substance frequently accumulate in the cell body. When the cytoplasm disintegrates, these inclusions are set free and remain between the other

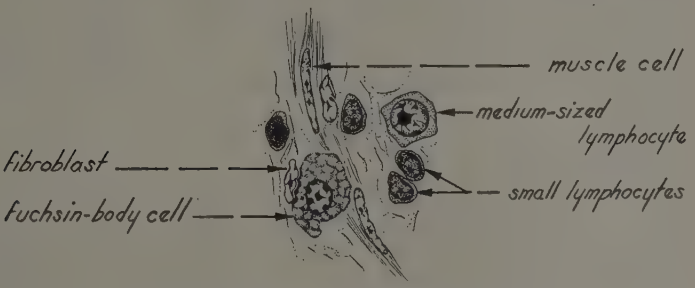


Fig. 8.—A leucocyte filled with fuchsin staining bodies, probably a young Russell body cell × 1280 (compare plate 7, *E*).

elements of the tissue as Russell bodies." Michels (1935), however, interprets these mulberrylike cells as erythrophages. He finds them abundantly in locations where erythrocytes have become extravasated and where plasma cells have made a heavy infiltration. In his opinion lymphocytes, monocytes, histiocytes, and endothelial cells, as well as plasma cells, may become erythrophages and hence are possible progenitors of Russell body cells. The variation in size of the Russell bodies is explained by assuming that the phagocytosed erythrocytes break into fragments, which then coalesce in varying degrees. Michels considers the Russell body cells an important aid to the hemoglobin-disintegrating reticuloendothelial system. In the turkey, however, their presence in scant numbers apparently precludes this interpretation.

The muscle fibers of the propria are typical smooth muscle cells, continuous with those of the muscularis mucosae.

Extravascular eosinophiles (fig. 3, *B;* plates 4, *C;* 5, *E;* 6, *A*, *B*) are numerous at the base of the tunica propria, diminishing in abundance near the orifice of the crypts. Two types of eosinophiles are present—one with a polymorphic nucleus and crystalloid eosinophilic granules, the

second usually with a nonlobate nucleus that may occasionally be polymorphic. The granules in this second type are larger than the crystalloids and are round. Both types of cells are found in the circulating blood of the turkey, the polymorphonuclear form occurring in the same relative abundance as the neutrophiles of mammals. A true neutrophile has not been observed in the turkey. Thus these two types of eosinophilic leucocytes of birds are comparable respectively with the neutrophiles and eosinophiles of mammals. Kasarinoff (1911) calls these forms "pseudo-eosinophiles" and "true eosinophiles." In the tunica propria the round granule type (true eosinophile) is far more numerous than the crystalloid form (pseudo-eosinophile).

In the turkey both types of eosinophiles are amphophilic after Susa's fixation; they stain lightly with eosin and intensely with hematoxylin. After Bouin's fixation the granular leucocytes of the propria are difficult to differentiate. After formalin fixation the granules have a fairly strong affinity for eosin but will retain hematoxylin unless carefully destained.

The hematoxylin staining gives the granules a true basophilic appearance which could easily lead one to misinterpret them as tissue mast cells. Jordan (1926), working with amphibia and teleosts, found basophilic cells to be numerous in the tunica propria, but was unable to consider them as mast cells. He believed them to be unripe, only partially ripe, or abortive degenerate eosinophiles. In the turkey they appear to be granular leucocytes that have migrated from the blood stream into the tunica propria. The pseudo-eosinophiles retain their round shape, and their crystalloid inclusions are morphologically identical with those of like cells in the blood stream. The true eosinophiles, however, do not remain round; their granules vary considerably in size but are always spherical. The reason for an altered staining affinity of eosinophiles located extravascularly in the propria is obscure.

The remaining portions of the duodenal wall are not complex in cellular detail and present no striking differences from mammalian tissues. The cells of all the muscle layers are identical in morphology and have the typical spindle shape of smooth muscle cells generally. Elastic fibers are present in all the various layers external to the tunica propria, being most abundant in the muscularis mucosae (plate 8, *B*) and in the connective tissues on either side of it. The elastic fibers are usually oriented in the same direction as the muscle fibers among which they are found and are always accompanied by abundant collagenous connective tissue. The subserosa is composed of connective tissue, principally of the collagenous type, but contains also numerous elastic fibers, chiefly disposed in a longitudinal fashion, with some placed circularly. The serosa consists

of a single layer of cells that forms a smooth covering for the viscera. The nuclei of the serosa cells are small and stain densely.

The enteric nerve plexuses, mentioned earlier in the paper, are histologically like those of mammals. The cells comprising the ganglia are generally seen in groups of four to eight in a 10-μ cross section of the intestine. They are large, measuring 14 × 18 μ, with a nucleus of 12-μ diameter. The nuclei are spherical with little chromatin; a single well-defined nucleolus occurs in each nucleus. In the cytoplasm, Nissl bodies are numerous. The ganglia are interconnected by nerve fibers.

THE DUODENUM OF THE POULT

The foregoing descriptions have been based on tissues from birds over eight months of age and here considered to be adults. A few observations have been made on younger birds—day-old and 5-months-old poults—and are recorded here for comparison with conditions obtaining in the older birds (see plate 1).

In a day-old poult the duodenal mucosa presents a regular picture of villus placement (plate, 1, *A*). On first examination one sees definite double rows of rounded villi, set in alternating positions to form rows that run the length of the intestine. If the intestine is opened and flattened, a space appears between the double rows in which, on further examination, a comparable double row of shorter villi is revealed. Thus there are double zigzagged rows of large villi, with similar rows of shorter villi in between. As the bird becomes older, the shorter villi grow out, and by the time it reaches maturity one can distinguish no differences between any of the villi (plate 1, *B, C*). The villi become more compressed and tonguelike as the bird matures.

In the poult the crypts of Lieberkühn are short, and the villi arise almost directly from the muscularis mucosae.

Lymph nodules do not occur in the tunica propria of the poult at hatching. At that time the propria is chiefly composed of fibroblasts, muscle fibers, and capillaries. A few lymphocytes are present. Some lymphocytic migration through villus epithelium occurs during the first day, and this becomes more common as the bird grows. By the end of two weeks lymphocytic aggregations are evident, and lymph nodules occur during the latter part of the first month of life. Eosinophiles are found in the propria, but not abundantly. Pseudo-eosinophiles do not occur early in the tunica propria. The muscularis mucosae and the longitudinal muscle layers are thin in the poult.

Goblet cells are numerous in the day-old turkey, but increase in relative numbers as the bird matures. This coincides with the finding (in *Gallus gallus domesticus*) of Ackert, Edgar, and Frick (1939), who be-

lieve the increase in goblet cells to be a factor in age resistance to parasitism, at least for the nematode *Ascaridia galli*[5] with which they were concerned.

[5] According to Beach and Freeborn (1936): "The name *Ascaridia galli* (Schrank, 1788) is accepted as the correct name for the European form of this worm, which was previously called *A. perspicillum* (Rudolphi, 1803). *Ascaridia lineata* (Schneider, 1866) was supposed to be the New World form. Recent comparisons of the two forms show no appreciable differences and hence the older name, *galli*, is used to include both forms."

LITERATURE CITED

Ackert, J. E., S. A. Edgar, and L. P. Frick.

1939. Goblet cells and age resistance of animals to parasitism. Amer. Micros. Soc. Trans. 58:81–89.

Beach, J. R., and S. B. Freeborn.

1936. Diseases and parasites of poultry in California. California Agr. Ext. Cir. 8:1–110. 5th ed.

Bizzozero, G.

1893. Ueber die schlauchförmigen Drüsen des magendarmkanals und die Beziehungen ihres Epithels zu dem Oberflächenepithel der Schleimhaut. Arch. f. Mikros. Anat. 42:136–37.

Calhoun, M. L.

1933. The microscopic anatomy of the digestive tract of *Gallus domesticus*. Iowa State Col. Jour. Sci. 7:261–382.

Ciaccio, C.

1907. Sopra speciali cellule granulose della mucosa intestinal. Arch. Ital. di Anat. e di Embriol. 6:482–98.

Clara, M.

1925. Beiträge zur Kenntnis des Vogeldarmes. I. Teil. Mikroskopische Anatomie. Ztschr. f. Mikros.-Anat. Forsch. 4:346–416.

1926*a*. Beiträge zur Kenntnis des Vogeldarmes. II. Teil. Die Hauptzellen des Darmepithels. Ztschr. f. Mikros.-Anat. Forsch. 6:1–27.

1926*b*. Beiträge zur Kenntnis des Vogeldarmes. III. Teil. Die basalgekörnten Zellen im Darmepithel. Ztschr. f. Mikros.-Anat. Forsch. 6:28–54.

1926*c*. Beiträge zur Kenntnis des Vogeldarmes. IV. Teil. Über das Vorkommen von Körnerzellen vom Typus der Panethschen Zellen bei den Vögeln. Ztschr. f. Mikros.-Anat. Forsch. 6:55–75.

1926*d*. Beiträge zur Kenntnis des Vogeldarmes. V. Teil. Die Schleimbildung im Darmepithel mit besonderer Berücksichtigung der Becherzellenfrage. Ztschr. f. Mikros.-Anat. Forsch. 6:256–304.

1926*e*. Beiträge zur Kenntnis des Vogeldarmes. VI. Teil. Das lympho-retikuläre Gewebe im Darmrohre mit besonderer Berücksichtigung der leukozytären Zellen. Ztschr. f. Mikros.-Anat. Forsch. 6:305–50.

1927*a*. Beiträge zur Kenntnis des Vogeldarmes. VII. Teil. Die Lieberkühn'schen Krypten. Ztschr. f. Mikros.-Anat. Forsch. 8:22–72.

1927*b*. Beiträge zur Kenntnis des Vogeldarmes. VIII. Teil. Das Problem des Rumpfdarmschleimhautreliefs. Ztschr. f. Mikros.-Anat. Forsch. 9:1–48.

Cordier, R.

1921. À propos des cellules argentaffines de l'intestin. Assoc. des Anat. [Paris] Compt. Rend. 16:61–68.

1923. Contribution à l'étude de la cellule de Ciaccio-Masson et de la cellule de Paneth. Soc. de Biol. [Paris] Compt. Rend. 88:1227–30.

Jordan, H. E.

1926. On the nature of the basophilic granulocytes of the blood and the tissues. Anat. Rec. 33:89–106.

1935. The significance of the lymphoid nodule. Amer. Jour. Anat. 57:1–37.

Kasakinoff, D.
1911. Experimentelle Blutuntersuchungen bei Vögeln. Folia Haematologia 10: 391–413.

Kaupp, B. F.
1918. Anatomy of the domestic fowl. 373 p. W. B. Saunders Company, Philadelphia.

Keasbey, L. E.
1923. On a new form of leucocyte (Schollenleukozyt, Weill) as found in the gastric mucosa of the sheep. Folia Haemotologia 29:155–71.

Kull, H.
1913. Die "basal gekörnten Zellen" des Dünndarmepithels. Arch. f. Mikros. Anat. 81:185–95.
1925. Die chromaffinen Zellen des Verdauungstraktes. Ztschr. f. Mikros.-Anat. Forsch. 2:163–200.

Kultschitzky, N.
1897. Zur Frage über den Bau des Darmkanals. Arch. f. Mikros. Anat. 49:7–35.

Latta, J. S.
1921. The histogenesis of dense lymphatic tissue of the intestine (Lepus): a contribution to the knowledge of the development of lymphatic tissue and blood-cell formation. Amer. Jour. Anat. 29:159–211.

Macklin, C. C., and M. T. Macklin.
1928. The intestinal epithelium. *In:* E. V. Cowdry. Special cytology. 2 vols., xx +1348 p. Paul B. Hoeber, Inc., New York. (See specifically vol. 1, p. 171–85.)

Masson, P.
1914. La gland endocrine de l'intestin chez l'homme. [Paris] Acad. des Sci. Compt. Rend. 158:59–61.
1928. Carcinoids (argentaffin-cell tumors) and nerve hyperplasia of the appendicular mucosa. Amer. Jour. Path. 4:181–212.

Masson, P., and L. Berger.
1923. Sur un nouveau mode de sécrétion interne: la neurocrinie. [Paris] Acad. des Sci. Compt. Rend. 176:1748.

Maximow, A. A., and W. Bloom.
1938. A textbook of histology. 3d ed. xiii + 668 p. W. B. Saunders Company, Philadelphia.

Michels, N. A.
1935. Medullary and non-medullary erythropoiesis with special reference to the plasma-cell eyrthrophage or Russell body cell, and to the erythrocatheretic (erythrolytic) function of lymph nodes and hemal nodes. Amer. Jour. Anat. 57:439–501.

Parat, M.
1924. Contribution à l'histophysiologie des organes digestifs de l'embryon. L'apparition corrélative de la cellule de Kultschitzky et de la secretine chez l'embryon. Soc. de Biol. [Paris] Compt. Rend. 90:1023–24.

Rosenberg, L. E.
1940. Post-mortem autodigestion of the intestinal mucosa of the turkey. Stain Tech. 15:53–56.

Schmidt, J. E.
1905. Beiträge zur normalen und pathologischen Histologie einiger Zellarten der Schleimhaut des menschlichen Darmkanales. Arch. f. Mikros. Anat. **66**: 12–40.

Simard, L. C., and E. V. Campenhaut.
1932. The embryonic development of argentaffin cells in the chick intestine. Anat. Rec. **53**:141–60.

Tang, E. H.
1922. Ueber die Panethschen Zellen sowie die gelben Zellen des Duodenums beim Schwein und den anderer Wirbeltieren. Arch. f. Mikros. Anat. **96**:182–209.

Weill, P.
1919. Ueber die leukocytaren Elemente der Darmschleimhaut der Säugtiere. Arch. f. Mikros. Anat. **93**:1–81.

TABLE AND PLATES

TABLE 1

Fixing, Staining, and Sectioning of Material Shown in Plates 1 to 8

Plate no.	Age of bird	Fixative	Stain	Thickness of sections in microns (μ)
1, *A*	1 day	10 per cent formol	None	—
1, *B*	5 months	10 per cent formol	None	—
1, *C*	1 year	10 per cent formol	None	—
2, *A–D*	1 year	10 per cent formol	Delafield's hematoxylin and eosin	10
3, *A*	8 months	Susa's	Masson's trichrome	10
3, *B*	8 months	Susa's	Heidenhain's hematoxylin and eosin	7
3, *C–D*	1 year	10 per cent formol	Delafield's hematoxylin and eosin	10
3, *E*	1 month	Bouin's	Delafield's hematoxylin and eosin	10
4, *A*	1 year	10 per cent formol	Delafield's hematoxylin and eosin	10
4, *B–C*	8 months	Susa's	Regaud's hematoxylin	10
4, *D*	1 year	10 per cent formol	Delafield's hematoxylin and eosin	10
5, *A*	8 months	Susa's	Regaud's hematoxylin and triosin	7
5, *B–C*	8 months	Susa's	Regaud's hematoxylin	10
5, *D*	1 month	Susa's	Regaud's hematoxylin	10
5, *E*	8 months	Susa's	Regaud's hematoxylin and triosin	7
6, *A*, *B*, and *D*	8 months	Susa's	Regaud's hematoxylin	10
6, *C*	8 months	Susa's	Masson's trichrome	10
7, *A*	8 months	Susa's	Regaud's hematoxylin	10
7, *B*	8 months	Susa's	Regaud's hematoxylin and triosin	7
7, *C*	1 year	10 per cent formol	Delafield's hematoxylin and eosin	10
7, *D*	8 months	Susa's	Heidenhain's hematoxylin and eosin	7
7, *E*	8 months	Susa's	Masson's trichrome	10
8, *A*	5 months	95 per cent alcohol	Delafield's hematoxylin and eosin	10
8, *B*	8 months	Bouin's	Delafield's hematoxylin light green and orcein	10
8, *C–D*	1 year	10 per cent formol	Delafield's hematoxylin and eosin	10

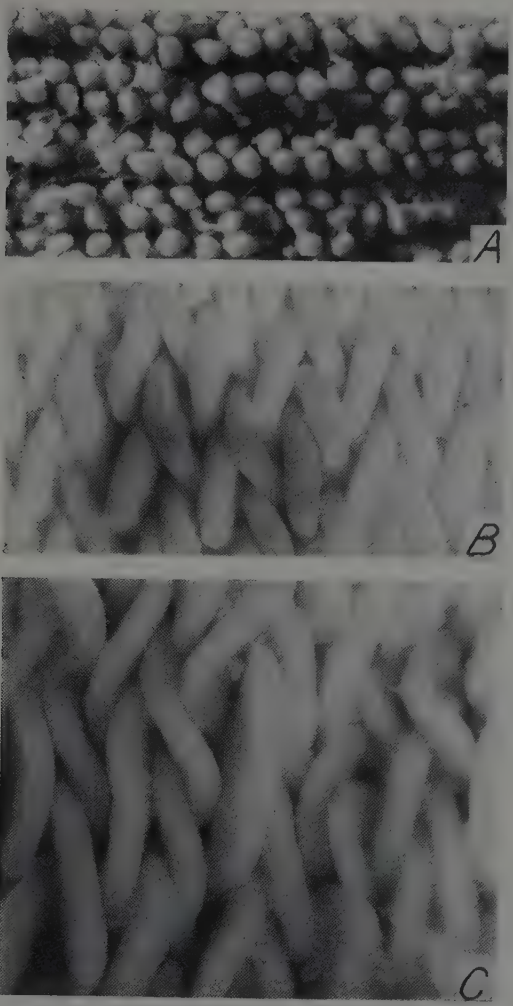
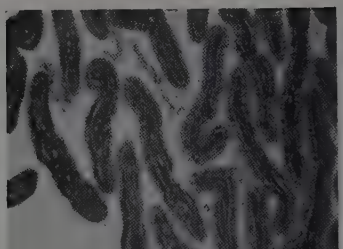
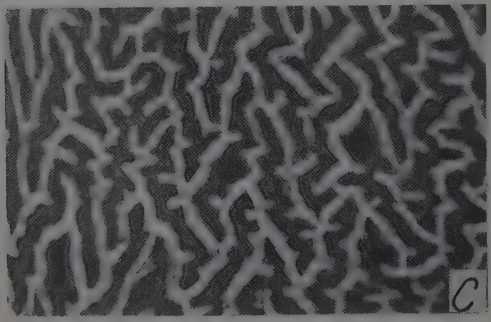


Plate 1.—Surface views of the duodenum (× 26) pinned open and immersed in formalin (photographs taken through the aqueous medium): *A*, tissue from a day-old turkey; *B*, from a five-months-old turkey; *C*, from a year-old turkey.





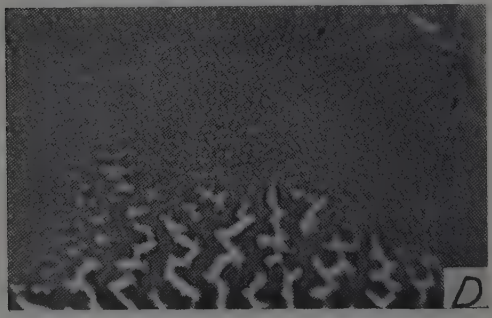
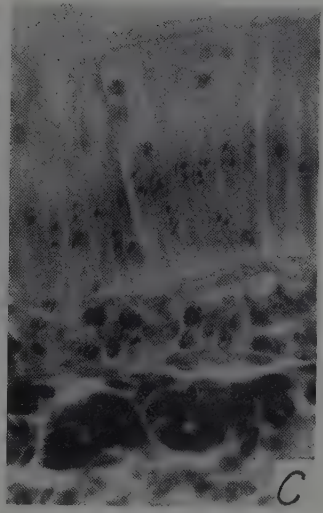


Plate 2.—Surface sections (× 26) of a small area of duodenum of an adult turkey to show the change in contour of the villi from their tips, *A*, to the crypts of Lieberkühn, *D*.



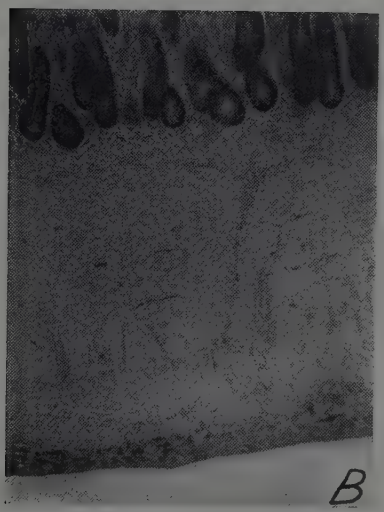


Plate 3.—*A*, Cross section (× 26) of duodenum of adult turkey illustrating the relative lengths of villi and the crypts of Lieberkühn (note the relatively thin muscular portion of the wall); *B*, the muscular wall (× 67); *C*, a portion of a villus cut longitudinally in which a blood capillary is shown separated by only a few cells from the membrana propria; *D*, chief cells and young goblet (mucous) cells of the villus epithelium; *E*, mature goblet cell of villus epithelium (*C*, *D*, and *E* × 640).

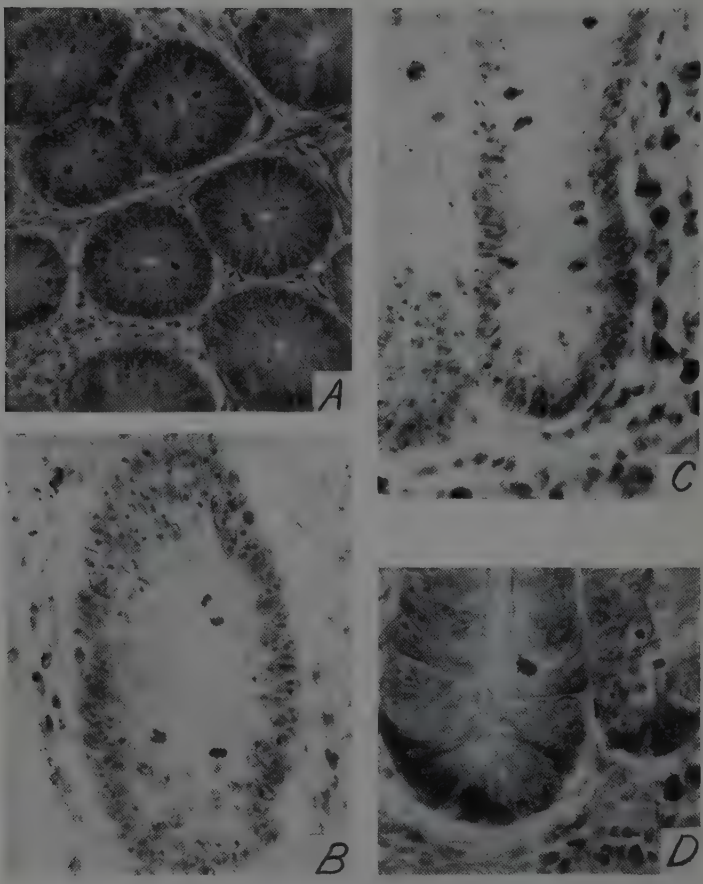


Plate 4.—*A*, Crypts of Lieberkühn in cross section, showing the abundant mitoses therein (× 300); *B*, *C*, and *D*, crypts in longitudinal section with mitotic figures of various stages (× 640).

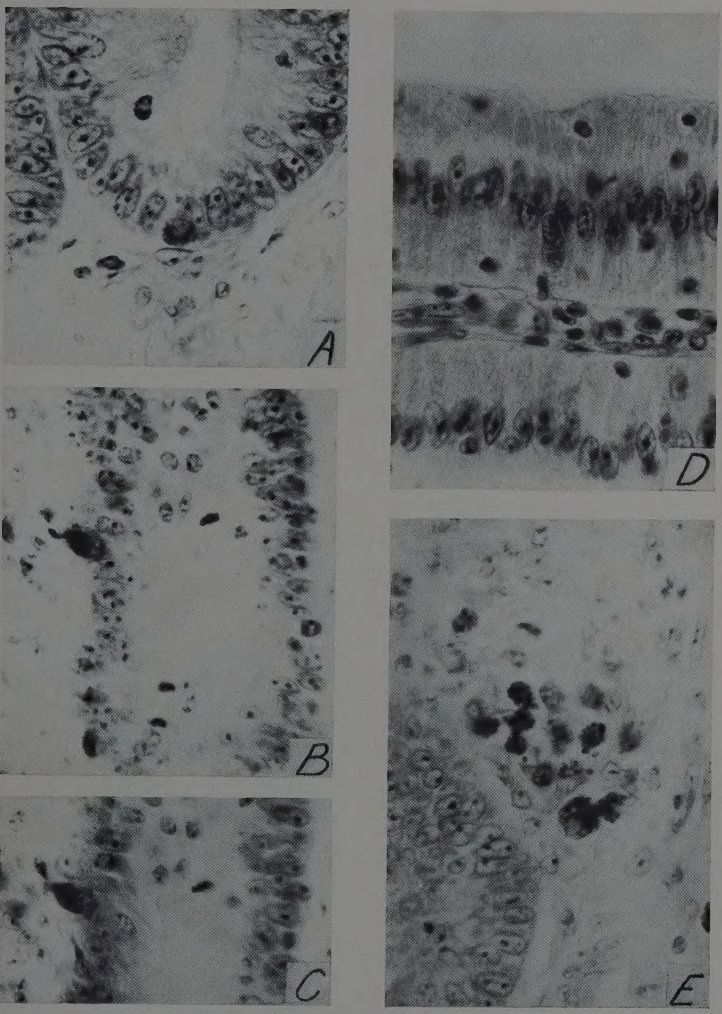


Plate 5.—Basal granular cells (× 640): *A*, basal part of a crypt in longitudinal section (compare text fig. 4, *B*). *B* and *C*, Middle portion of a crypt with migrating lymphocyte penetrating the membrana propria at the base of a basal granular cell (compare text fig. 4, *A*). *D*, Villus epithelium in longitudinal section with a similar phenomenon (compare text fig. 3, *A*). *E*, Basal granular cell free in the tunica propria (compare text fig. 3, *B*).

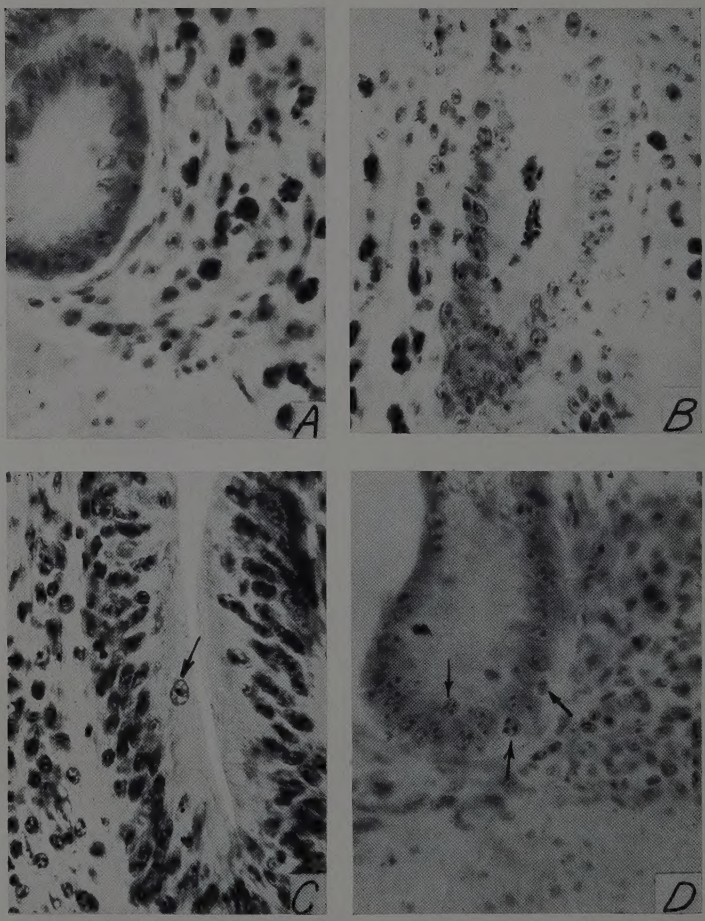
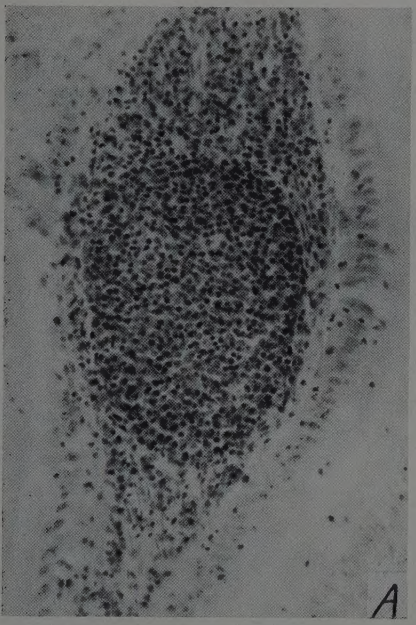
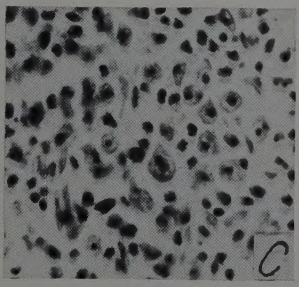
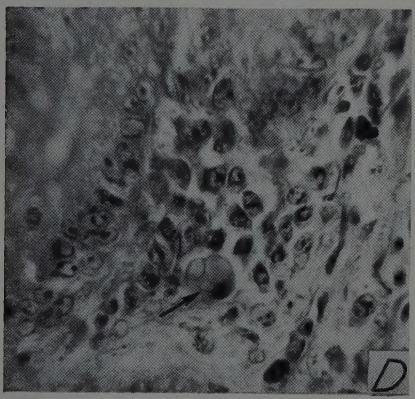


Plate 6.—Longitudinal sections of the crypt region (× 640): *A*, free eosinophiles in the tunica propria; *B*, a section similar to *A*, but two eosinophiles have migrated into the lumen of the crypt; *C*, a large lymphocyte migrating through the crypt epithelium; *D*, schollen-leucocytes in the crypt epithelium (compare text fig. 6).







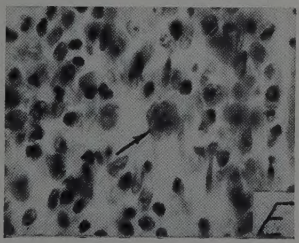
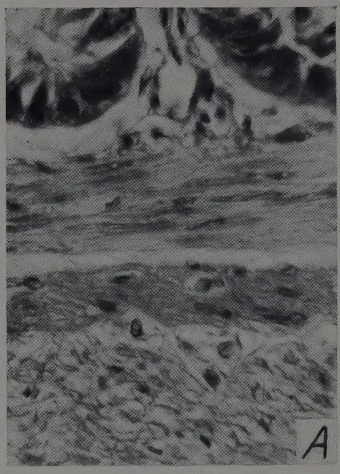
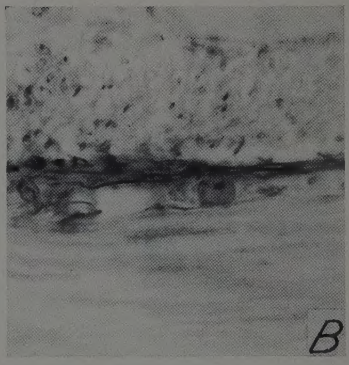


Plate 7.—*A*, Lymph nodule of a villus cut longitudinally (× 300); *B*, the same (× 640). *C*, Lymphocytes of the tunica propria (× 640) from the middle portion of a villus (compare text fig. 5). *D*, Russell body cell and neighboring elements (× 640) in the tunica propria of the crypt region (compare text fig. 7). *E*, A cell filled with granules (× 640) which stain with fuchsin, probably a young Russell body cell (compare text fig. 8).





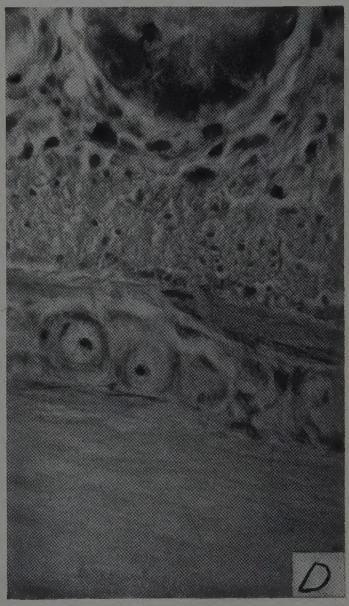


Plate 8.—*A*, A longitudinal section of duodenum showing the accessory layer of circular muscle between the muscularis mucosae and the circular muscle of the muscularis. *B*, A cross section of duodenum showing the distribution of elastic fibers in the connective tissue between muscularis mucosae and the accessory circular muscle. *C*, A cross section of nerve of Auerbach's plexus located in the longitudinal muscle of the muscularis. *D*, Ganglion cells of Meissner's plexus as seen in a cross section of the duodenum. (All figures × 640).

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